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Purification and Properties of Agmatine Ureohydrolyase, a Putrescine Biosynthetic Enzyme in *Escherichia coli*

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The putrescine biosynthetic enzyme agmatine ureohydrolase (AUH) (EC 3.5.3.11) catalyzes the conversion of agmatine to putrescine in *Escherichia coli*. AUH was purified approximately 1,600-fold from an *E. coli* strain transformed with the plasmid pKA5 bearing the *speB* gene encoding the enzyme. The purification procedure included ammonium sulfate precipitation, heat treatment, and DEAE-sephacel column chromatography. The molecular mass of nondenatured AUH is approximately 80,000 daltons as determined by gel-sieving column chromatography, while on denaturing polyacrylamide gels, the molecular mass is approximately 38,000 daltons; thus, native AUH is most likely a dimer. A radiolabeled protein extracted from minicells carrying the pKA5 plasmid comigrated with the purified AUH in both sodium dodecyl sulfate-polyacrylamide and native polyacrylamide gels. The pI of purified AUH is between 8.2 and 8.4, as determined by either chromatofocusing or isoelectric focusing. The K_m of purified AUH for agmatine is 1.2 mM; the pH optimum is 7.3. Neither the numerous ions and nucleotides tested nor polyamines affected AUH activity in vitro. EDTA and EGTA [ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid] at 1 mM inactivated AUH activity by 53 and 74%, respectively; none of numerous divalent cations tested restored AUH activity. Ornithine inhibited AUH activity noncompetitively ($K_i = 6 \times 10^{-3}$ M), while arginine inhibited AUH activity competitively ($K_i = 9 \times 10^{-3}$ M).

Putrescine, the biosynthetic precursor of spermidine, is produced in *Escherichia coli* by either of two pathways (21). Pathway I involves the decarboxylation of ornithine; pathway II involves the decarboxylation of arginine to agmatine, followed by the removal of urea from agmatine by agmatine ureohydrolase. Regulation of the biosynthetic enzymes if complex (16, 20); their regulation involves both transcriptional and posttranslational mechanisms (3, 13, 14, 25, 26, 29, 30). Although the intracellular level of putrescine is constant as well as proportional to the growth rate (4), the relative use of the two polyamine biosynthetic pathways can vary considerably (23, 26), depending on the nutritional supplements provided. The biosynthetic forms of ornithine decarboxylase (EC 4.1.1.17) (1) and arginine decarboxylase (EC 4.1.1.19) (31) have been purified and characterized; however, little is known about the physical and biochemical properties of agmatine ureohydrolase (AUH) (EC 3.5.3.11). In this paper, we describe the purification and some properties of AUH. We examined the effects of nucleotides, precursors and products of agmatine metabolism, and various ions on AUH activity. In addition, the K_m, thermal stability, pH, and temperature optima were determined.

MATERIALS AND METHODS

Strains. *E. coli* K-12 HT328 (Δ*speA* Δ*speB* Δ*speC*) was obtained from H. Tabor, National Institutes of Health, Bethesda, Md.

Plasmids. pKA5 was obtained from D. Markham, Fox Chase Cancer Research Center, Philadelphia, Pa. Plasmid pKA5 encodes arginine decarboxylase, agmatine ureohydrolase, and methionine adenosyltransferase. Plasmid pKA13 encodes arginine decarboxylase and methionine adenosyltransferase. These plasmids are hybrids of pBR322 and the putrescine biosynthetic genes derived from the *E. coli* genome (5, 28). Transformation was carried out by the method of Dagert and Ehrlich (8).

Bacterial cultures. Purification of AUH was carried out on extracts prepared from *E. coli* HT328 transformed with pKA5. Cultures used for innoculation were grown overnight in Luria broth (19) with glucose (0.2%) and ampicillin (50 μg/ml) at 37°C in a reciprocal shaking waterbath. An overnight culture of strain HT328 transformed with pKA5 was used to innoculate 18 liters of Luria-glucose medium to an optical density of 0.02 to 0.05 (measured at 575 nm).

Materials. All chemicals (reagent grade) and other supplies were obtained from either Sigma Chemical Co., Aldrich Chemical Co., Boehringer-Mannheim, Pharmacia Fine Chemicals, or Difco Laboratories. The radiochemicals were obtained from Amersham Corp. Nonradiolabeled protein markers were obtained from Pharmacia.

AUH assays. A sensitive radioactive assay (22) was refined and used to monitor AUH activity during the course of purification. Release of $^{14}CO_2$ from L-[guanido-^{14}C]arginine was measured by trapping the CO_2 in filter paper wicks saturated with Protosol (New England Nuclear Corp.). The reactions were incubated at 37°C, and each reaction mixture (0.3 ml) contained 100 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.8), 0.04 mM pyridoxal phosphate, 4 mM $MgSO_4$, 1 mM dithiothreitol, 2 mM L-arginine, 10 mM L-agmatine, 5 μCi of L-[guanido-^{14}C]arginine, 20 U of soybean urease (Sigma), and 50 U of arginine decarboxylase (ADC) incubated for 30 min. A dialyzed crude extract of *E. coli* HT328 transformed with pKA13 was concentrated to 7 to 10 mg of protein per ml in a colloidon bag (cutoff, 25 kilodaltons; Schleicher & Schuell, Inc.) and used as the source of ADC. The transformed strain, when grown in Luria broth with glucose, had a 12- to

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15-fold increase in ADC activity when compared with other *E. coli* K-12 strains carrying a single gene for ADC.

By the radioactive assay, the specific activity of AUH detected in a strain bearing a chromosomal copy of *speB* was approximately 1 to 2 μmol/min per mg of protein; in a strain bearing pKA5, the specific activity of AUH was 13 μmol/min per mg of protein; in a strain deleted for *speB*, the specific activity of AUH was not detectable (less than 0.01 mol/min per mg of protein). No AUH activity was detectable in the absence of added ADC or urease. To study the kinetics of AUH and to evaluate the role of ions, nucleotides, precursors and products of agmatine metabolism, and chelating agents, the radioactive assay was modified. The substrate L-[guanido-^{14}C]arginine was decarboxylated by incubation with the crude ADC preparation; after 3 h, 70 to 80% arginine was decarboxylated as monitored by a parallel assay of $^{14}CO_2$ released from L-[U-^{14}C]arginine. The protein in the cocktail was denatured by incubation in a boiling waterbath for 10 min and then removed by centrifugation at 12,000 × *g* for 15 min. The deproteinized cocktail was used for AUH assays. In those assays assessing the effects of various metal ions, radioactive agmatine was prepared in the absence of Mg^{2+}; this was possible as ADC still possessed 30% of its decarboxylating activity. A parallel assay of $^{14}CO_2$ released from arginine was used to estimate the amount of arginine added because of the lack of conversion to agmatine. In some experiments, the colorimetric assay for AUH (26) was used to substantiate the results obtained with the radioactive assay.

ADC. The ADC assay used was as described by Wu and Morris (31).

Enzyme activity. Protein was determined by the method of Bradford (6). Specific activities of ADC and AUH were defined in micromoles of CO_2 liberated per minute per milligram of protein. One unit of urease is defined as 1 μmol of NH_3 generated (from urea) per min at pH 7.0 and 25°C.

Gel electrophoresis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 10% acrylamide with the buffer system containing 0.1% SDS as described by Laemmli (17). Before loading, the protein samples were heated at 80°C for 5 min in 0.01 M Tris hydrochloride buffer (pH 7.4) containing 0.1% SDS, 0.14 M β-mercaptoethanol, and 10% glycerol. After the electrophoresis, the gels were removed and stained with Coomassie blue (17) or silver nitrate (27). Radiolabeled protein bands were detected by the method of Bonner and Laskey (2).

Elution of AUH from polyacrylamide gels. Native PAGE (9) of the purified preparation was carried out at 4°C. The 12-cm 5% gels were sliced into 1-mm sections and eluted overnight in breakage buffer, and the sections and buffer were assayed for AUH activity. Radioactivities in radiolabeled proteins in gel sections were determined by the alkaline digestion method as described by Long (18). Minicell preparation and labeling of plasmid-encoded proteins were performed as described by Boyle et al. (5).

Column chromatography. The gels for ion exchange, molecular sieving, and chromatofocusing were prepared, packed, and run as recommended by the manufacturer (Pharmacia). DEAE-sephacel and chromatofocusing fractions were collected in tubes containing a final concentration of 1 mM agmatine sulfate (pH 7.0) while Sephadex G-100 and Sephacryl S-200 columns were eluted with buffer containing 1 mM agmatine sulfate (pH 7.0). After the pHs of the chromatofocused fractions were determined 1 M Tris hydrochloride (pH 7.5) was added to a final concentration of 0.10 M, and then AUH activity was assayed.

Antiserum production. Antibodies to purified AUH and to DEAE-Sephacel fractions were prepared in New Zealand White male rabbits by the method of Clausen (7). Ouchterlony analysis was performed in 1% agarose–0.05 M Tris hydrochloride (pH 7.5)–0.1 M NaCl.

Purification steps. (i) Crude extracts. All manipulations of harvested bacteria and cell extracts were performed at approximately 4°C. *E. coli* HT328 transformed with pKA5 was cultured as described earlier. The culture, when it reached an optical density (at 575 nm) of 1.5, was harvested by centrifugation at 10,000 × *g* for 10 min and washed twice with 0.1 M HEPES (pH 7.4); the bacterial pellets were suspended in breakage buffer (0.1 M HEPES [pH 7.4], 5 mM $MgCl_2$, 5 mM dithiothreitol, 0.04 mM pyridoxal phosphate, 1 mM agmatine) to 1/50 the volume of the original culture. Breakage was achieved by passing the cell suspension twice through an Aminco pressure cell at 10,000 lb/in^2. The resulting extract was sonicated at 4/10 the scale with a Virsonic cell disruptor (model 16-850) for 1 min with a fine tip probe. Cell debris and unbroken cells were removed by two successive centrifugations at 12,000 × *g* for 20 min.

(ii) Ammonium sulfate precipitation. The crude extract was diluted with 0.1 M HEPES (pH 7.4) to adjust the final protein concentration to 5 mg/ml. A 90% ammonium sulfate solution (saturated in 0.1 M HEPES-sodium hydroxide [pH 7.4]) was added to the crude extract at the rate of 35 ml/h with constant stirring to reach 33% ammonium sulfate saturation (values refer to percent saturation at 25°C). The mixture was allowed to stand for 4 h at 4°C. The precipitate formed was removed by centrifugation at 12,000 × *g* for 20 min and discarded. Further dropwise addition of a 90% solution of ammonium sulfate to the supernatant of the previous step to give a final saturation of 48% resulted in the precipitation of AUH activity. The precipitate was collected as described above and washed with a 48% saturated ammonium sulfate solution in 0.1 M HEPES (pH 7.4). The precipitate from the above procedure was suspended in 100 ml of a 40% ammonium sulfate solution in 0.1 M HEPES containing 1 mM agmatine (pH 7.4) and slowly stirred overnight at 4°C. The precipitate was collected by centrifugation at 12,000 × *g* for 20 min and washed with a 40% ammonium sulfate solution in 0.1 M HEPES (pH 7.4).

The precipitate from this ammonium sulfate cut was suspended in 100 ml of 0.05 M Tris hydrochloride (pH 7.5) and dialyzed for 16 h against 2 4-liter changes of 0.1 M Tris hydrochloride (pH 7.5) containing 1 mM agmatine. The insoluble material was removed by centrifugation at 12,000 × *g* for 30 min and discarded.

(iii) Heat treatment. The dialyzed ammonium sulfate fraction was heated for 10 min at 65°C. Coagulated proteins were removed by centrifugation at 12,000 × *g* for 30 min.

(iv) 1 M Tris adjustment. The supernatant of the heat stable fraction was adjusted to 1 M Tris hydrochloride (pH 7.5) by adding 2 M Tris hydrochloride (pH 7.5). The solution was allowed to stand for 1 h at 4°C, and the precipitate was removed by centrifugation at 12,000 × *g* for 30 min. The supernatant was dialyzed for 16 h against 2 4-liter changes of 0.01 M Tris hydrochloride (pH 7.5).

(v) DEAE-Sephacel column chromatography. The sample from the Tris treatment and dialysis was applied to a column of DEAE-sephacel (packed volume, 130 ml) preequilibriated with 10 mM Tris hydrochloride (pH 7.5). The column was washed with 130 ml of 200 mM Tris hydrochloride (pH 7.5). The enzyme activity eluted with 500 ml of the same buffer in which the Tris concentration was increased linearly from 200 to 600 mM at a flow rate of 1 ml/min. The fractions with

TABLE 1. Summary of purification of AUH

Purification step	Total activity (U)[a]	Total protein (mg)	Sp act	Purification (fold)	% Yield
Crude extract	11,131	1,084.9	10.26	1	100
Ammonium sulfate precipitation	8,074	272.4	29.68	4	73
Heat treatment	7,668	26.3	291	41	69
1 M Tris adjustment	7,241	14.7	512	74	65
DEAE-Sephacel chromatography (pH 7.5)	22,144	3.09	7,166	351	199
Sephadex G-100 column chromatography	27,932	2.14	13,052	507	251
Chromatofocusing	23,627	0.68	34,745	1,595	212

[a] One unit of activity is 1 nmol of CO_2 formed per h.

AUH activity (approximately 0.55 M Tris) were pooled. A colloidon bag (cutoff, 25 kilodaltons; Shleicher & Schuell) was used to concentrate the fractions to 5 mg of protein per ml and to equilibriate the sample to 0.15 M Tris hydrochloride (pH 7.4).

(vi) Sephadex G-100 column chromatography. AUH fractions concentrated after DEAE-sephacel column chromatography were applied to a Sephadex G-100 column and were eluted at a rate of 25 ml/h (loading volume, ≤1% of packed gel volume). The molecular weight with respect to elution volume for the standard proteins (Pharmacia) were determined.

(vii) Chromatofocusing. The dialyzed ammonium sulfate fraction was further dialyzed twice for 16 h against 4 liters of 0.025 M imidazole hydrochloride (pH 7.4) starting buffer. This sample was applied to an ion-exchange column for chromatofocusing (Polybuffer exchanger; Pharmacia) equilibriated with the starting buffer. The column was eluted with polybuffer 74 (pH 4.0; Pharmacia).

RESULTS

AUH purification. Washed cell pellets were often stored at −20°C for 14 weeks without any significant loss of AUH activity. No appreciable loss of AUH activity in crude extracts was observed after storage at −20°C for 4 weeks and for up to 12 weeks at −80°C if 1 mM agmatine and 10% glycerol were included in the buffer. Purified AUH was approximately twofold more stable in the presence of 1 mM agmatine or 10% glycerol or both at −80, −20, and 4°C; the purified enzyme was stable for at least 3 months at −80°C. Mercaptoethanol at 0.1 mM completely inactivated crude or purified AUH in 3 days at 4°C in the presence of 1 mM agmatine.

The seven steps used to purify AUH resulted in 1,600-fold purification with a greater than 100% recovery (Table 1). This recovery is presumably due to the removal of some inhibitors(s) after DEAE-Sephacel chromatography, which resulted in a threefold activation of the enzyme.

Nature of AUH inhibitor. Because of the threefold activation of AUH after DEAE-Sephacel chromatography of a 1 M Tris adjustment (Table 1), it was of interest to determine whether an inhibitor of AUH was present. When an ammonium sulfate fraction was chromatofocused, we observed in mixing experiments that pooled AUH activity in fractions with a pI of 8.0 to 8.5 was inhibited approximately 80% by pooled fractions with a pI between 4.7 to 5.5 when mixed in a 1:1 ratio. To further investigate the nature of this inhibitory activity, portions of the pooled fractions with a pI of 4.7 to 5.5 were treated with RNase (100 μg/ml for 30 min at 37°C), with proteinase K (250 μg/ml for 30 min at 37°C) and then 1 mM phenylmethylsulfonyl flouride, or with heat (100°C for 10 min). These pretreated inhibitory fractions were mixed with the pooled AUH fractions at a ratio of 9:1 (wt/vol) and incubated, and the AUH activity was assayed by the radiochemical method (see Materials and Methods). The results observed are strongly suggestive that the inhibitory substance is proteinaceous as its inhibitory activity towards AUH was reduced 80% by the heat or proteinase K treatments but was unaffected by RNase treatment.

Effects of various metals, chelating agents, nucleotides, and precursors and products of agmatine metabolism on AUH activity. None of the following compounds had a significant effect on the activity of AUH as measured by the radiochemical method: Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Na^{2+}, K^{+}, Fe^{2+}, ATP, GTP, CTP, TTP, AMP, GMP, 3′, 5′-cyclic AMP, 2′,3′-cyclic AMP, 3′,5′-cyclic GMP, guanosine-5′,3′-bisdiphosphate, glutamate, glutamine, methionine, putrescine, and spermidine (1 to 10 mM). Ornithine inhibited AUH activity in a noncompetitive mode ($K_i = 6 \times 10^{-3}$M), while arginine inhibited in a competitive mode ($K_i = 9 \times 10^{-3}$ M). Similar inhibition results were obtained by using the colorimetric assay for AUH. Both EDTA and EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], at a concentration of 1 mM, irreversibly inactivated the enzyme by 53 and 74%, respectively, in the presence of absence of 1 mM agmatine. Addition of chlorides, sulfates, and acetates of magnesium, manganese, zinc, calcium, sodium, potassium, copper, and zinc at concentrations of up to 10 mM to an EDTA-treated AUH preparation before or after dialysis did not restore catalytic activity. This suggests that AUH requires a metal ion for its structural stability rather than its catalytic activity.

Enzyme parameters. Urea formation (determined by radiochemical or colorimetric assays) was linear with time for 75 min at pH 7.5 and 37°C. The optimum pH was between 7.2 and 7.4 for maximum activity at 37°C. The optimum temperature of incubation for maximum activity at pH 7.4 was 42°C. Agmatine was an absolute requirement for the preservation of crude enzyme activity during heating at 65°C. Activity was linear for up to 500 μg of protein per assay in crude extracts and up to 13 μg per assay with the purified preparation eluted from the chromatofocusing column. AUH exhibited Michaelis-Menten kinetics for agmatine with apparent K_m values of 1.2 mM by radioactive assay and 1.9 mM by the colorimetric assay.

Physical properties and enzyme purity. The molecular mass of purified native AUH determined by gel seiving chromatography was approximately 80 ± 5 kilodaltons. The molecular mass (Fig. 1) of AUH by SDS-PAGE was calculated to be 38 kilodaltons. The purified AUH (Fig. 1, lane g) comigrated with the putative radiolabeled AUH from minicell proteins (lane i) encoded by the plasmid pKA5 (5). The enzyme preparation was approximately 98% homogeneous on the gel stained with Coomassie brilliant blue (Fig. 2). After silver staining, two minor protein bands representing about 5 to 10% of the total material were detected; they had molecular masses of 70 and 24 kilodaltons. The 70-kilodalton protein was present in all five separate preparations of purified AUH; however, the 24-kilodalton protein was not always present.

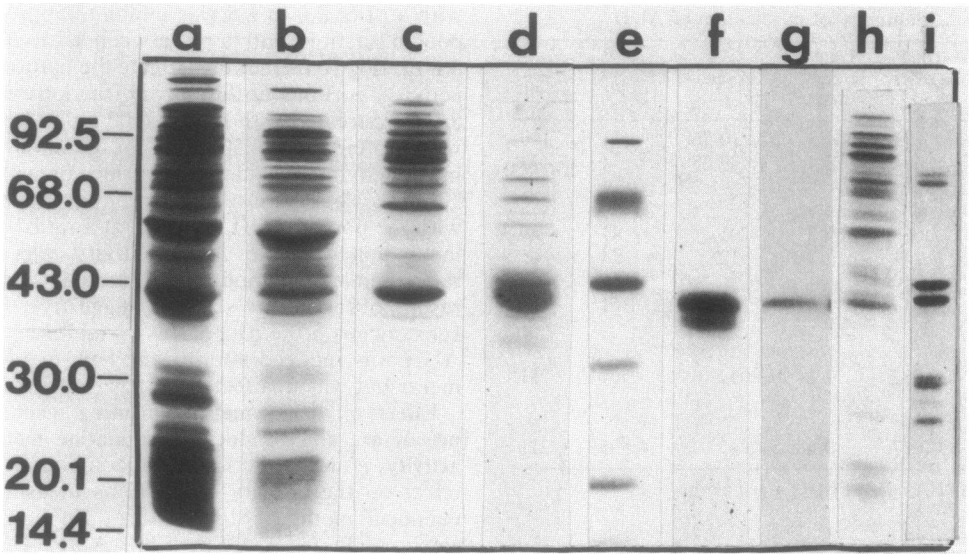

FIG. 1. SDS-PAGE of the purified enzyme or protein fractions from the various steps of purification containing AUH activity or pKA5-encoded, [^{35}S]methionine-labeled proteins from minicells. Lane a, Crude extract. Lane b, Ammonium sulfate precipitation. Lane c, DEAE-Sephacel (pH 7.5). Lane d, Sephacryl S-200. Lane e, Molecular weight standards (in thousands): phosphorylase b, 92.5; bovine serum albumin, 68; ovalbumin, 43; α-chymotrypsinogen, carbonic anhydrase, 30; soybean trypsin inhibitor, 20.1; alpha-lactalbumin, 14.4. Lane f, Chromatofocusing of ammonium sulfate fraction (polybuffer 74). Lane g, Chromatofocusing of Sephadex G-100 fraction (polybuffer 96). Lane h, DEAE-Sephacel (pH 8.5). Lane i, pKA5-encoded proteins labeled with ^{35}S]methionine in minicells. The protein bands were stained with Coomassie brilliant blue. Labeled proteins in lane i were detected by autoradiography.

When analyzed by a native PAGE system for basic proteins (see Methods), the activity of purified AUH comigrated with a major radiolabeled protein from minicells containing pKA5 (Fig. 2, bottom) as well as with the major Coomassie blue-stained protein in a purified AUH preparation (Fig. 2, top). These observations, combined with those shown in lanes g and i of Fig. 1, identify the 38-kilodalton radiolabeled protein encoded by pKA5 as being AUH.

Chromatofocusing and electrophoretic mobility. When a dialyzed ammonium sulfate fraction was chromatofocused, AUH activity was recovered in fractions with a pI range of 5.3 to 5.8 (Fig. 3b). When an ammonium sulfate fraction was

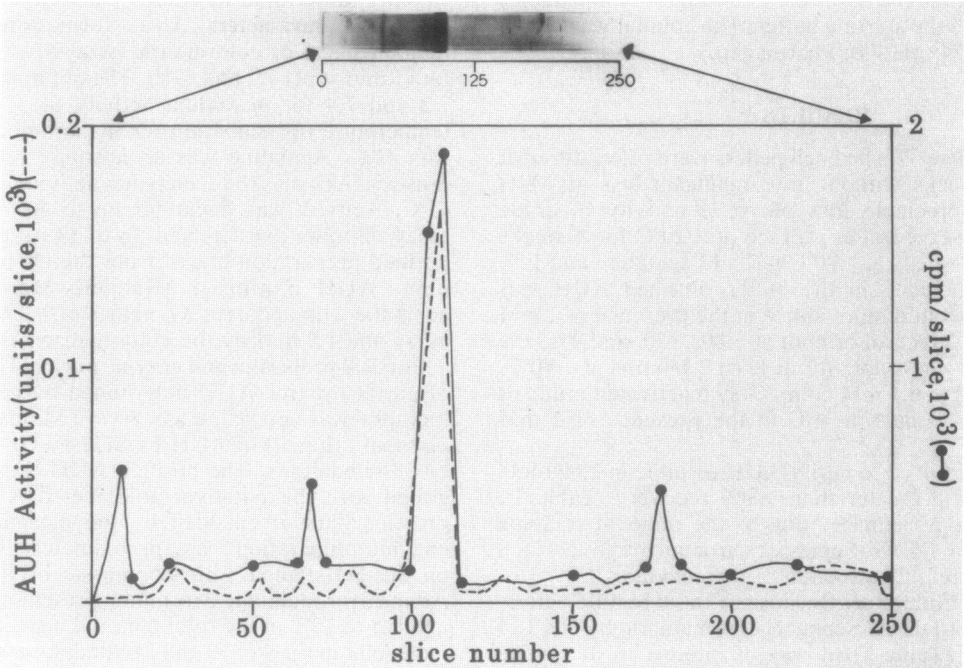

FIG. 2. (Top) Polyacrylamide gel electrophoresis of 15 μg of purified AUH stained with Coomassie brilliant blue. (Bottom) Radioactivity and enzyme activity in gel slices of native PAGE on [^{35}S]methionine-labeled proteins in minicells bearing the pKA5 plasmid and 15 μg of purified enzyme.

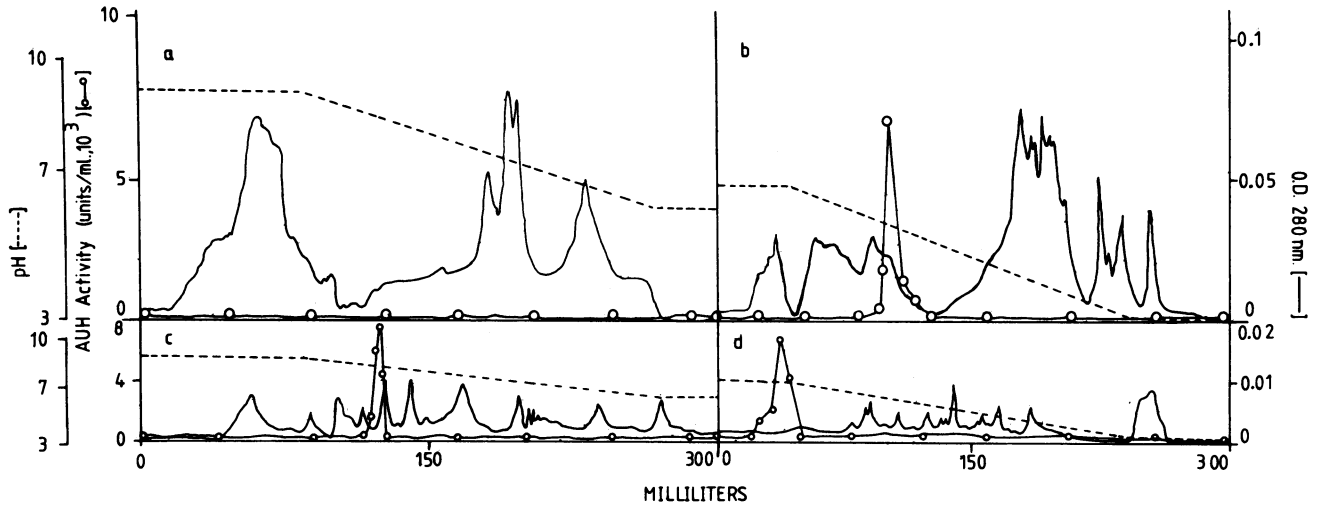

FIG. 3. Elution profile of AUH on chromatofocusing column. (a) Ammonium sulfate fraction with polybuffer 96. (b) Ammonium sulfate fraction with polybuffer 74. (c) Sephadex G-100 fraction with polybuffer 96. (d) Sephadex G-100 fraction with polybuffer 74.

dialyzed against 0.025 M ethanolamine hydrochloride (pH 9.4) and applied and eluted with polybuffer 96 (pH 7.0; Pharmacia), no AUH activity was recovered from the column (Fig. 3a). An AUH assay on the flowthrough from the sample loading demonstrated that AUH did not bind to the chromatofocusing column at pH 7.0 (data not shown). These results suggest that crude AUH is an acidic protein with a pI somewhere between 5 and 6.

Because we had demonstrated the presence of a proteinaceous inhibitor, it was decided to determine if the removal of this inhibitory fraction would alter the pI of AUH. When a Sephadex G-100 fraction was dialyzed against 0.025 M ethanolamine hydrochloride (pH 9.4) and applied and eluted with polybuffer 96, AUH activity was recovered in fraction with a pI range of 8.2 to 8.4 (Fig. 3c). In contrast, when the G-100 fraction was dialyzed against as well as applied with 0.025 M imidazole hydrochloride (pH 7.4) and then eluted with polybuffer 74 (pH 4.0), AUH activity was recovered in the unbound fraction. It appears that Sephacel chromatography causes the dissociation of a protein component(s) from AUH, which in turn results in a net change in the pI of AUH, i.e., from a pI of ~5.5 for crude AUH to a pI of ~8.3 for purified AUH.

Isoelectric focusing and two-dimensional gel analyses of [^{35}S]methionine-labeled proteins from crude extracts of minicells carrying the plasmid pKA5 demonstrated that AUH had a pI of approximately 5.0 (data not shown) and confirmed the chromatofocusing data results obtained with AUH activity from an ammonium sulfate cut.

Antigenic properties. Preincubation of rabbit antiserum with purified enzyme for 2 h at 37°C blocked enzyme activity by 92% when compared with controls treated with preimmune serum. Reaction of the antiserum with the purified AUH or various purified fractions resulted in a single immunoprecipitation band on Ouchterlony plates (data not shown). We did not detect an immunoprecipitable band in the extracts from *E. coli* deleted for *speB*; this corroborates the genetic evidence that this region of the chromosome is a complete deletion (12).

DISCUSSION

The native form of purified AUH in *E. coli* appears to be a dimer whose subunit molecular weight is approximately 38,000, as judged by activity profiles on gel filtration columns and mobility on denaturing gels. Purified enzyme, when fractionated by Sephacryl S-200 chromatography, separated into two distinct protein peaks of 40 and 80 kilodaltons. AUH activity was associated with only the 80-kilodalton peak. Because a heat treatment step was used in the purification, it was possible that the purified AUH preparation was partially degraded but still active. However, the AUH activity profiles of ammonium sulfate fractions and purified enzyme preparations were not significantly different when run on either a Sephacryl A-200 column or a DEAE-Sephacel column (data not shown). Moreover, there was an enrichment of the 38-kilodalton protein during purification (Fig. 1). These data suggest that the heat treatment did not cause any significant degradation of AUH.

AUH underwent a noticeable activation (Table 1) during purification; this activation can be explained by the removal of an inhibitory protein after DEAE-Sephacel chromatography. This possibility is supported by two observations: (i) the change in the pI of AUH from 5.5 to 8.3 after purification and (ii) the decrease in AUH inhibitory activity associated with the chromatofocused fractions (pI, 4.5 to 5) after proteinase and heat treatments. This copurification of an inhibitory protein is similar to that described in purifying ornithine decarboxylase, the other putrescine biosynthetic enzyme in *E. coli*. An acidic protein inhibitor, termed ornithine decarboxylase antizyme, was found to copurify with ornithine decarboxylase in the initial stages of purification (16).

Both ornithine and arginine decarboxylase in *E. coli* are subject to end product inhibition by physiological concentrations of putrescine and spermidine in vitro (1, 32). In contrast, this study shows that AUH was not inhibited by these polyamines in vitro. This lack of end product inhibition in vitro is consistent with our observations in vivo. Putrescine supplementation of *E. coli* did not cause repression of AUH activity but did cause repression of ornithine and arginine decarboxylase activities (26). It is interesting to note that the agmatine precursors, ornithine and arginine, inhibit AUH activity. The inhibition by ornithine may be involved in posttranslational control of AUH or pathway selection of putrescine synthesis from ornithine as opposed to arginine (20), or it may be involved in both. However, in

the absence of any demonstrable effectors which would lower the K_m of AUH for agmatine (1.2 to 1.9 mM), this possibility at present seems remote, as the intracellular concentration of ornithine in *E. coli* is in the range of 0.03 to 0.04 mM (23). The competitive inhibition by arginine is probably not of regulatory significance in vivo because of two factors: (i) the 10 mM concentration of arginine required for significant inhibition relative to the estimated intracellular arginine concentration of 0.3 to 0.4 mM (23); and (ii) the reported competition between the guanido groups of small organic compounds and agmatine on agmatinase activity (15); i.e., the guanido group of arginine probably competes with that on agmatine in a nonspecific manner.

Agmatinase has been detected in gram-negative bacteria but not in gram-positive bacteria (24). The enzyme from *Proteus vulgaris* was purified 50-fold with a pH optimum of 9.3 and a K_m for agmatine of 0.7 mM. The native enzyme had a molecular weight of 150,000 as determined by gel sieving. *P. vulgaris* and *Klebsiella aerogenes* can use agmatine as carbon and nitrogen sources because they have an inducible agmatinase (10, 11). AUH in *E. coli* is also induced by agmatine (26), although the extent of induction reported was one-fifth that of observed in *Klebsiella* or *Proteus* spp.; furthermore, agmatine could not be used as a source of carbon or nitrogen or both by *E. coli* (26). Compared with that in *P. vulgaris*, the AUH in *E. coli* has a fourfold-lower specific activity and a twofold higher K_m for agmatine. This information, combined with results from our previous studies (26) which suggested the absence of biodegradative AUH in *E. coli*, is consistent with a role for AUH in the production of putrescine as a cellular component rather than as a source of carbon or nitrogen or both.

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