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# Arginine Decarboxylase from a *Pseudomonas* Species

H. J. ROSENFELD* AND J. ROBERTS
*Memorial Sloan-Kettering Cancer Center, New York, New York 10021*

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An arginine decarboxylase has been isolated from a *Pseudomonas* species. The enzyme is constitutive and did not appear to be repressed by a variety of carbon sources. After an approximately 40-fold purification, the enzyme appeared more similar in its properties to the *Escherichia coli* biosynthetic arginine decarboxylase than to the *E. coli* inducible (biodegradative) enzyme. The *Pseudomonas* arginine decarboxylase exhibited a pH optimum of 8.1 and an absolute requirement of $Mg^{2+}$ and pyridoxal phosphate, and was inhibited significantly at lower $Mg^{2+}$ concentrations by the polyamines putrescine, spermidine, and cadaverine. The $K_m$ for L-arginine was about 0.25 mM at pH 8.1 and 7.2. The enzyme was completely inhibited by *p*-chloromercuribenzoate. The inhibition was prevented by dithiothreitol, a feature that suggests the involvement of an -SH group. Of a variety of labeled amino acids tested, only L-arginine, but not D-arginine, was decarboxylated. D-Arginine was a potent inhibitor of arginine decarboxylase with a $K_i$ of 3.2 $\mu$M.

The existence in *Escherichia coli* of two distinct arginine decarboxylases has been convincingly demonstrated. The inducible arginine decarboxylase requires a complex amino acid mixture and a low pH in the growth medium for its synthesis and exhibits maximal activity at pH 5.2 (1). In contrast, the biosynthetic arginine decarboxylase, which is involved in polyamine biosynthesis, has a much less stringent growth medium requirement and exhibits a pH optimum of 8.4 (10). Both enzymes require pyridoxal phosphate (PALP), but only the biosynthetic decarboxylase has an absolute requirement for $Mg^{2+}$.

In this laboratory, numerous known bacteria and soil isolates were tested for an arginine-degrading enzyme that could lower plasma arginine when injected into animals and be suitable for studying the effect of arginine deprivation on growth of tumors. One organism, a *Pseudomonas* species called *Pseudomonas* XA, was found to contain an arginine decarboxylase that in preliminary experiments appeared to lower plasma arginine when injected into mice. The present report describes the partial purification and characterization of the *Pseudomonas* arginine decarboxylase.

## MATERIALS AND METHODS

**Isolation, characterization, and growth of the microorganism.** The organism was isolated from a local stagnant pond, by selective enrichment on L-arginine as the sole carbon and nitrogen source (3), and has been classified as a *Pseudomonas* species. It is a short, gram-negative, motile bacillary organism and occurs primarily singly but somtimes in pairs. Optimal growth temperature is about 30 C. In broth cultures, it forms a sediment and thin pellicle. On blood agar plates, colonies are circular, raised, and greenish-gray. Hemolysis is evident on blood agar plates, and a gelatin stab is liquefied rapidly. The organism does not hydrolyze starch or produce $H_2S$ or indole, but it is catalase and oxidase positive. Acid is produced from glucose, but no acid or gas is produced from sucrose, mannitol, lactose, sorbitol, rhamnose, arabinose, or maltose. Ammonia is formed during growth in peptone broth. A yellow-green fluorescent pigment is observed in *Pseudomonas* F agar (Difco). After 48 h, litmus milk becomes acidic and a clot forms.

The guanine-plus-cytosine content was found to be 63.3 mol% by buoyant density.

Bacteria were grown routinely in batch culture, 1.5 liters of medium in a 6-liter flask, shaken vigorously at 30 C in a New Brunswick Gyrotory incubator shaker. A minimal medium was used, containing per liter deionized water: $KH_2PO_4$, 2 g; L-arginine, 10 g; and 100×-concentrated salts solution (8), 10 ml. The pH was adjusted to 7.0 with NaOH prior to autoclaving.

*E. coli* UW44, furnished by D. Morris, University of Washington, Seattle, was grown in the minimal medium described by Wu and Morris (10).

**Preparation of cell extracts.** Enzyme isolation and purification were conducted at 0 to 7 C. Harvested cells were washed and suspended in 2 volumes of 50 mM potassium phosphate (pH 7.2) containing 1 mM dithiothreitol (DTT) and 0.02 mM PALP. Cells were sonically disrupted with a Bronwill Biosonik IV with three 3-min bursts. The extract was centrifuged

for 30 min at 20,000 × *g*, and the supernatant fluid was stored at −80 C.

**Enzyme assay.** Decarboxylase activity was determined by trapping and counting $^{14}CO_2$ released from [1-$^{14}$C]arginine as described previously (4, 10). The reaction mixture contained: 100 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.5, or 8.4; 5 mM $MgSO_4$; 1 mM DTT; 0.04 mM PALP; 7 mM L-[1-$^{14}$C]arginine, specific radioactivity usually 0.003 mCi/mmol; and enzyme to yield a total volume of 0.5 ml. Reactions were incubated at 37 C for 30 min with gentle agitation. One enzyme unit represents 1 μmol of $CO_2$ produced per 1 min under the conditions of the assay. Enzyme specific activity is expressed as enzyme units per milligram of protein. Protein was determined by the method of Lowry et al. (6), with bovine serum albumin as the standard.

**Preparation of L-[1-$^{14}$C]arginine.** Only DL-[1-$^{14}$C]arginine was available commercially (Amersham/Searle). However, it was discovered that D-arginine inhibited markedly the decarboxylation of the L isomer. Hog kidney D-amino acid oxidase (Sigma Chemical Co.) served to resolve the racemic mixture. An overnight incubation at room temperature was necessary to destroy completely the D isomer as measured by production of $NH_3$. Column chromatography with Bio-Rex 70 separated L-arginine from "α-keto-arginine." The latter compound was eluted immediately by passing water through the column. L-Arginine was bound strongly to the resin and could be released by stepwise elution with 0.25 M potassium phosphate, pH 7.4.

**Materials.** L-Homoarginine and agmatine were purchased from Aldrich Chemical Co. L-Canavanine was from Calbiochem. L-Citrulline was supplied by Eastman. J. Berger of Hoffmann-LaRoche kindly supplied $N^5$-hydroxy-DL-arginine. All other amino acids were purchased from Sigma Chemical Co. DL-[1-$^{14}$C]arginine was obtained from Amersham/Searle.

**Thin-layer chromatography.** Cellulose-coated aluminum sheets (E. Merck) were used to separate arginine, agmatine, and putrescine. The most useful solvent for separation of these substances was the following alkaline mixture: 1-butanol-methylethylketone-acetone-methanol-28% $NH_4OH$-water (4:2:2:0.2:0.5:1.4, by volume). The compounds were visualized by spraying with ninhydrin (in 2-propanol), followed by heating at 100 C for several minutes.

## RESULTS

**Influence of various growth media on arginine decarboxylase levels.** *Pseudomonas* XA was grown on several different carbon sources to determine whether arginine decarboxylase was inducible and/or repressible. Ammonium sulfate, at 0.4%, was present in all media, and all carbon sources were at 1%. The organism was unable to grow on glucose alone, and sodium succinate or sodium lactate permitted significant bacterial growth only after 48 h (an overnight incubation was sufficient for the other cultures). Growth on each single carbon source resulted in an arginine decarboxylase activity that was just slightly less than that achieved with arginine alone (Table 1). Furthermore, little or no repression of the enzyme was produced by any of the tested carbon sources. Snell medium (1), which was developed to attain maximal inducible arginine decarboxylase synthesis in *E. coli*, permitted reasonable growth of *Pseudomonas* XA. These cells contained no inducible arginine decarboxylase, as determined by the absence of $CO_2$ evolution at pH 5.2, but did synthesize decarboxylase (activity at pH 7.4) at a level equivalent to that observed in bacteria from defined media (Table 1). Thus, *Pseudmonas* XA produces an apparently constitutive arginine decarboxylase that is not repressed by the compounds tested.

**Partial purification of arginine decarboxylase. (i) Ammonium sulfate fractionation.** Approximately 125 ml of crude sonic extract was subjected to ammonium sulfate fractionation at 0 to 4 C. The salt was added slowly while the pH was maintained between 7.0 and 7.2 with dilute $NH_4OH$. Three fractions were prepared: 0 to 25%, 25 to 50%, and 50 to 80% saturation (calculated for 0 C). Each pellet was dissolved with 10 ml of 40 mM potassium phosphate (pH 6.8; containing 1 mM $MgSO_4$, 0.1 mM DTT, and 0.05 mM PALP) and then dialyzed overnight against 4 liters of the same buffer, with one buffer change. Most of the enzyme activity (85% of the total recovered in the three fractions) was present in the middle fraction. No increase in specific activity, however, was associated with this fraction (Table 2).

**(ii) Diethylaminoethyl-Sephadex column**

TABLE 1. *Influence of growth medium on arginine decarboxylase activity*

| Growth medium | Cell wet wt[a] (g/100 ml) | Arginine decarboxylase sp act[b] |
|---|---|---|
| L-Arginine | 0.60 | 0.0031 |
| D-Glucose | 0 | |
| Arginine + glucose | 0.80 | 0.0029 |
| L-Asparagine | 0.61 | 0.0023 |
| Arginine + asparagine | 0.69 | 0.0021 |
| Sodium succinate | 0.45 | 0.0024 |
| Arginine + sodium succinate | 0.52 | 0.0025 |
| Sodium lactate | 0.39 | 0.0022 |
| Arginine + sodium lactate | 0.74 | 0.0028 |
| Snell medium | 0.30 | 0.0027 |

[a] Cells were harvested in late-log phase, where it was determined that the enzyme-specific activity was optimal.

[b] Enzyme units per milligram of protein.

TABLE 2. *Summary of purification steps for arginine decarboxylase*

| Purification step | Vol (ml) | Protein (mg/ml) | Enzyme (units/ml) | Recovery (%) | Sp act[a] | Purification (fold) |
|---|---|---|---|---|---|---|
| Sonic extract | 125 | 17.2 | 0.053 | | 0.0031 | |
| 25–50% Ammonium sulfate | 35 | 38.2 | 0.122 | 64 | 0.0032 | |
| Diethylaminoethyl-Sephadex | 250 | 2.0 | 0.0178 | 67 | 0.0089 | 2.9 |
| Carboxymethyl-Sephadex | 127 | 0.16 | 0.0094 | 18 | 0.059 | 19 |
| Sephadex G200 | 25 | 0.34 | 0.038 | 14 | 0.113 | 37 |

[a] Enzyme units per milligram of protein.

**chromatography.** Resin (A-50 grade, Pharmacia Fine Chemicals, Inc.) was equilibrated with the above-mentioned buffer and then poured into a column (2.5-cm diameter) to a height of 80 cm. The dialyzed 25 to 50% ammonium sulfate fraction was applied, and elution was effected by a 0 to 0.7 M KCl linear gradient in the phosphate buffer (1 liter in each reservoir). Fractions (10 ml each) were collected; the initial column flow rate was about 25 ml/h. The decarboxylase was eluted at 0.3 M KCl, as determined by a conductivity bridge. Tubes with enzyme activity (25 fractions) were pooled, dialyzed overnight against 5 mM potassium phosphate buffer, and lyophilized. About a threefold purification was attained by this chromatography (Table 2).

**(iii) Carboxymethyl-Sephadex column chromatography.** Resin (C-50 grade, Pharmacia) was equilibrated with 20 mM sodium acetate (pH 6.4; containing $Mg^{2+}$, DTT, and PALP at the concentrations stated above) and then poured into a column (2.5-cm diameter) to a height of 50 cm. The lyophilized enzyme was dissolved in 10 ml of water and then dialyzed exhaustively against the sodium acetate buffer. Before the sample was applied to the column, the pH was adjusted to 6.4. A 50-ml volume of eluting starting buffer was first passed through the loaded column, and then eluting was effected by a 0 to 0.1 M NaCl linear gradient in sodium acetate buffer (500 ml in each reservoir). The initial flow rate was 30 ml/h. Owing to the presence of a contaminating enzyme activity in some of the active fractions (agmatine ureohydrolase and urease), which caused $^{14}CO_2$ evolution from L-[*guanido*-$^{14}$C]arginine, a considerable fraction of the recovered arginine decarboxylase activity was sacrificed. The pH of the pooled enzyme was raised to about 7.5, and then the enzyme was dialyzed overnight against 5 mM potassium phosphate buffer (pH 7.5) containing $Mg^{2+}$, DTT, and PALP. The pool was finally lyophilized.

**(iv) Sephadex G200 column chromatography.** The lyophilized enzyme from the carboxymethyl-Sephadex step was dissolved in 5 ml of cold water and dialyzed for 5 h against 1 liter of 40 mM potassium phosphate (pH 7.5) with cofactors. The enzyme was then chromatographed on a G200 column (1.5 by 55 cm). The flow rate was 20 ml/h; each fraction contained 2 ml. Appropriate fractions were combined, dialyzed against 5 mM phosphate buffer, and lyophilized. This preparation, which was nearly 37-fold purer than the sonic extract, was used in subsequent studies to characterize *Pseudomonas* XA arginine decarboxylase.

**pH optimum of arginine decarboxylase.** Five different buffers, each at a final concentration of 0.1 M, were used to cover the pH range 5 to 10. The precise pH in each case was determined by constructing simulated reaction mixtures (minus enzyme, or 25 $\mu$g of protein per assay) and measuring the pH at 37 C. The pH optimum was quite sharp, showing a maximum at pH 8.1 (Fig. 1). This value is close to that reported for *E. coli* constitutive (biosynthetic) arginine decarboxylase, i.e., pH 8.4 (10), and different from the inducible (biodegradative) arginine decarboxylase of *E. coli*, which has a pH optimum of 5.2 (1).

**Estimation of arginine decarboxylase $K_m$.** The $K_m$ for L-arginine was determined at pH 8.1 (the pH optimum) and at a more physiological value, pH 7.2. HEPES buffer was used in both cases. To restrict the extent of reaction to less than 10%, incubation time was 10 min rather than 30 min, and only 1/10 the usual enzyme level was employed. To compensate for these two changes, the L-[1-$^{14}$C]arginine specific activity was raised from 0.003 to 0.2 $\mu$Ci/$\mu$mol. $K_m$ values were calculated from the data of Fig. 2 (note the different scales for the ordinate at the 2 pH values). Nearly identical $K_m$ values were obtained: 0.23 mM (at pH 7.2) and 0.26 mM (at pH 8.1). However, the $V_{max}$ at pH 8.1 was four times greater than that at pH 7.2. An approximately 10-times-lower $K_m$ has been reported for the *E. coli* constitutive arginine decarboxylase (10).

**Substrate specificity and inhibition of ar-**

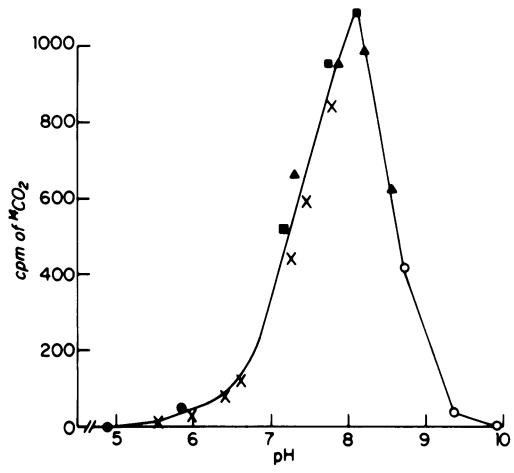


FIG. 1. *pH optimum of arginine decarboxylase. A standard reaction mixture as described in the text was employed, substituting only the buffer (at 0.1 M, final concentration) to obtain the desired pH. Symbols: ●, sodium acetate; ×, potassium phosphate; ▲, tris(hydroxymethyl)aminomethane; ○, glycine-KCl; ■, HEPES. The most highly purified enzyme was used.*

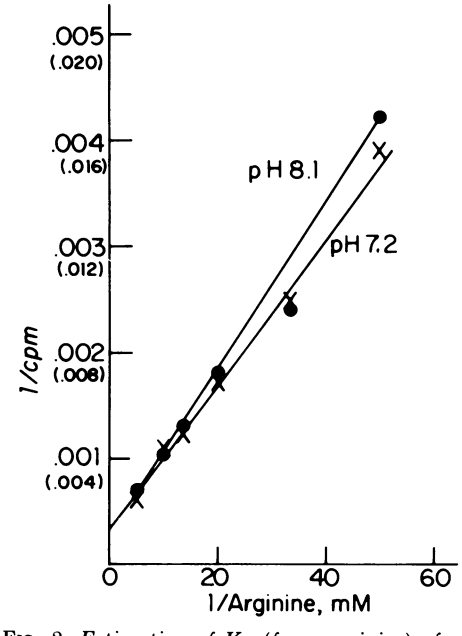


FIG. 2. *Estimation of $K_m$ (for L-arginine) of arginine decarboxylase, at two different pH values. Specific radioactivity of L-[1-$^{14}$C]arginine was increased to 0.20 Ci/mol, incubation was at 37 C for 10 min, and only 1/10 the usual amount of enzyme (most highly purified) was added. The scale in parentheses on the ordinate pertains to values for the study at pH 7.2. Symbols: ×, pH 7.2; ●, pH 8.1.*

**ginine decarboxylase by related compounds.** The substrate specificity of partially purified arginine decarboxylase was evaluated by incubating it with several $^{14}$C-labeled amino acids, either uniformly labeled or carboxyl labeled. Citrulline, ornithine, lysine, phenylalanine, tryptophan, and arginine were tried. Only L-arginine, but not D-arginine, yielded significant amounts of $^{14}CO_2$, indicating high substrate specificity (data not shown).

A variety of compounds that are either analogues of L-arginine or related metabolically to L-arginine was tested for the ability to inhibit arginine decarboxylase (Table 3). Citrulline, ornithine, and lysine showed little or no effect, even when present at a concentration 10 times greater than arginine. Moderate inhibition was seen in the case of $N^5$-hydroxyarginine, homoarginine, and canavanine. D-Arginine was a very potent inhibitor of L-arginine decarboxylation even when the D-arginine was present at a

TABLE 3. *Inhibition of arginine decarboxylase by related compounds*[a]

| Inhibitor | Concn (mM) | % Inhibition |
|---|---|---|
| D-Arginine | 0.7 | 79 |
| | 7 | 99 |
| L-Citrulline | 7 | 0 |
| | 70 | 0 |
| L-Ornithine | 7 | 0 |
| | 70 | 27 |
| L-Lysine | 7 | 0 |
| | 70 | 20 |
| $N^5$-hydroxy-DL-arginine | 7 | 46 |
| L-Homoarginine | 1.4 | 14 |
| | 3.5 | 27 |
| | 7 | 47 |
| L-Canavanine | 1.4 | 12 |
| | 3.5 | 21 |
| | 7 | 30 |
| Agmatine | 7 | 48 |
| | 21 | 75 |
| Putrescine | 7 | 0 |
| | 70 | 82 |
| Spermidine | 7 | 10 |
| | 21 | 67 |

[a] Assays performed at pH 8.1 with the most highly purified enzyme; L-[1-$^{14}$C]arginine was 7 mM, and $MgSO_4$ was 5 mM.

concentration 10 times less than the L isomer. The $K_i$ for D-arginine was determined in the experiment described below. Product inhibition was evident since an equimolar mixture of L-arginine and its immediate decarboxylation product, agmatine, produced about 50% inhibition of the reaction. This effect was due to agmatine and not its hydrolysis product, putrescine, because the purified enzyme used in these assays contained only a trace of agmatine ureohydrolase (verified by thin-layer chromatography). The polyamines putrescine and spermidine caused significant inhibition of arginine decarboxylase only at elevated polyamine concentrations. This phenomenon was examined further in relation to the $Mg^{2+}$ concentration.

**Effect of $Mg^{2+}$ concentration on inhibition of arginine decarboxylase by polyamines.** Wu and Morris (10) have claimed that inhibition of the biosynthetic *E. coli* arginine decarboxylase by polyamines is diminished by $Mg^{2+}$. Since the inhibition studies described above were performed under standard conditions (5 mM $Mg^{2+}$), it was decided to investigate further this relationship with the *Pseudomonas* XA enzyme. As is evident from Table 4, inhibition of *Pseudomonas* XA arginine decarboxylase by putrescine and spermidine was overcome by a higher $Mg^{2+}$ concentration. This was especially apparent at the lower polyamine levels. Qualitatively similar results were obtained with agmatine, although only one concentration was tested. Cadaverine, the decarboxylation product of lysine, is another polyamine that exhibited a similar response (Table 4). Thus, inhibition of arginine decarboxylase by polyamines in not pathway specific.

TABLE 4. *Effect of $Mg^{2+}$ on inhibition of arginine decarboxylase by polyamines*

| Polyamine | Concn (mM) | % Inhibition of arginine decarboxylase[a] | | |
|---|---|---|---|---|
| | | 1 mM $Mg^{2+}$ | 2 mM $Mg^{2+}$ | 5 mM $Mg^{2+}$ |
| Agmatine | 7 | 64 | 49 | 40 |
| Putrescine | 7 | 25 | 10 | 8 |
| | 17.5 | 64 | 37 | 22 |
| | 35 | 92 | 78 | 56 |
| Spermidine | 2 | 40 | 11 | 4 |
| | 5 | 69 | 34 | 15 |
| | 10 | 93 | 75 | 44 |
| Cadaverine | 2 | 20 | 8 | 0 |
| | 5 | 30 | 19 | 14 |
| | 10 | 55 | 35 | 23 |
| | 20 | 75 | 65 | 48 |

[a] Assays performed at pH 8.1 with the most highly purified enzyme; L-[1-$^{14}$C]arginine was 7 mM.

**Estimation of $K_i$ for D-arginine.** In view of the striking ability of D-arginine to inhibit decarboxylation of L-arginine, it was desirable to determine whether the D isomer was itself a substrate for the decarboxylase. By means of thin-layer chromatography, it was observed that incubation of L-arginine with purified enzyme resulted in the appearance of an agmatine spot concomitant with the disappearance of substrate; however, incubation of D-arginine with enzyme for up to 90 min resulted in no detectable conversion. Since D-arginine did not serve as a substrate for arginine decarboxylase, the $K_i$ for this inhibitor was determined by the method of Dixon and Webb (2). Figure 3 depicts the resulting plot. The $K_i$ for D-arginine was calculated to be 3.2 $\mu$m. The point of intersection did not fall on the abscissa, suggesting that the inhibition is competitive. Since a mixed-type inhibition yields a similar Dixon plot, the data of Fig. 3 were replotted as slope versus $1/S$. The resulting straight line passed essentially through the origin (graph not shown), again indicating that D-arginine is a competitive inhibitor.

**Effect of general enzyme inhibitors on arginine decarboxylase.** In preliminary experiments, it was found that partially purified enzyme that had been dialzyed in the absence of $Mg^{2+}$ and PALP lost more than 95% of its activity. When the dialyzed enzyme was reconstituted with $Mg^{2+}$ and PALP, approximately 40% of the activity was recovered. Table 5 summarizes experiments in which specific in-

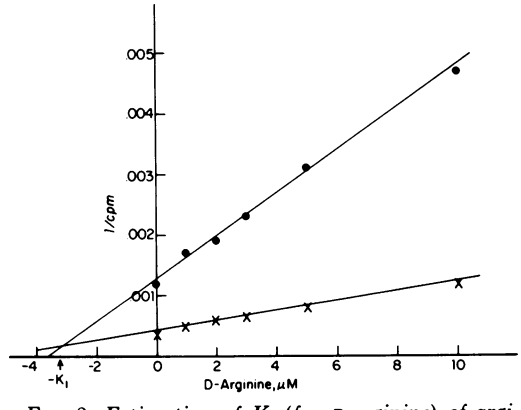


FIG. 3. *Estimation of $K_i$ (for D-arginine) of arginine decarboxylase. The modified protocol outlined in the legend of Fig. 2 was followed, but only at pH 8.1. The purest enzyme was used. Symbols: ×, L-arginine at 0.1 mM; ●, L-arginine at 0.02 mM.*

TABLE 5. *Inhibition of arginine decarboxylase by general enzyme inhibitors*

| Supplements | % Inhibition of arginine decarboxylase[a] | | |
|---|---|---|---|
| | 1 mM EDTA | 0.1 mM PCMB | 7 mM AOA |
| None | 93 | 100 | |
| 1 mM $Mg^{2+}$ | 25 | 100 | |
| 5mM $Mg^{2+}$ | 0 | | |
| 0.1 mM DTT | 92 | 7 | |
| 1 mM DTT | | 0 | |
| 1 mM $Mg^{2+}$ + 0.1 mM DTT | 34 | 0 | |
| 5 mM $Mg^{2+}$ + 0.1 mM DTT | 0 | | |
| 1 mM $Mg^{2+}$ + 1 mM DTT | | 3 | |
| 5 mM $Mg^{2+}$ + 1 mM DTT | | | 100 |

[a] Assays performed at pH 8.1 with the most highly purified enzyme; L-[1-$^{14}$C]arginine was 7 mM, and PALP, at 0.04 mM, was present in all assays. EDTA, Ethylenediaminetetraacetate; PCMB, *p*-chloromercuribenzoate; AOA, aminooxyacetic acid.

hibitors were utilized to study cofactor requirement. All incubations contained 40 $\mu$M PALP and 0.2 mM $Mg^{2+}$, which were introduced unavoidably by addition of purified enzyme. The nearly complete inhibition of decarboxylase by 1 mM ethylenediaminetetraacetate was overcome by progressively greater quantities of $Mg^{2+}$. Similarly, 0.1 mM *p*-chloromercuribenzoate inhibited the enzyme completely and DTT could reverse this effect. In the presence or absence of PALP, 7 mM aminooxyacetic acid, a potent inhibitor of vitamin $B_6$-dependent enzymes, prevented completely the degradation of arginine. Thus, arginine decarboxylase of *Pseudomonas* XA had an absolute requirement for $Mg^{2+}$ and PALP and apparently requires a free —SH group for full activity.

## DISCUSSION

The isolate *Pseudomonas* XA contains an arginine decarboxylase that, on the basis of its nearly invariant activity in different media, may be considered a constitutive enzyme. Of interest is the observation that *Pseudomonas* XA was unable to synthesize an inducible arginine decarboxylase in Snell medium, which, in *E. coli*, permits the formation of optimal levels of the inducible enzyme. The rather low pH (6.0) of the culture in Snell medium may explain the abnormally low growth yield in such a rich medium. It is thought that the function of inducible decarboxylases may be to counteract a low pH in the medium.

Of numerous $^{14}$C-labeled amino acids tested as the substrate (phenylalanine, tryptophan, citrulline, ornithine, and lysine), only L-arginine, but not D-arginine, yielded significant quantities of $^{14}CO_2$, indicating a highly specific decarboxylase. However, D-arginine was a potent inhibitor of L-arginine decarboxylation. In its kinetic properties, the *Pseudomonas* XA arginine decarboxylase is more similar to the biosynthetic arginine decarboxylase of *E. coli* than to the biodegradative enzyme (Table 6). The *Pseudomonas* XA and *E. coli* biosynthetic arginine decarboxylases have similar pH optima and cofactor requirements. Also, as noted above (Table 4), they are similar in the antagonistic effect of $Mg^{2+}$ in relieving polyamine-mediated inhibition. However, this phenomenon is not pathway specific since cadaverine also inhibits the enzyme. A significant kinetic dissimilarity between these two enzymes is in their $K_m$ values for arginine. As a control, the $K_m$ value for the *E. coli* biosynthetic enzyme was determined in parallel with that for *Pseudomonas* XA, and the published value of 0.030 mM was confirmed. Nearly a 10-times-higher $K_m$ value was observed for the *Pseudomonas* XA enzyme.

During enzyme purification, a second arginine-degrading activity was detected. The contaminant activity was expressed as $^{14}CO_2$ release from L-[*guanido*-$^{14}$C]arginine and was presumed to be arginine dihydrolase. However, this assumption proved to be incorrect because the observed activity could be explained by the combined action of agmatine ureohydrolase and

TABLE 6. *Comparison of properties of several arginine decarboxylases*

| Organism | $K_m$ for arginine (mM) | pH optimum | $Mg^{2+}$ requirement | PALP |
|---|---|---|---|---|
| *E. coli* (inducible)[a] | 0.65 | 5.2 | No | Yes |
| *E. coli* (constitutive)[a] | 0.030 | 8.4 | Yes | Yes |
| *Pseudomonas* XA | 0.25 | 8.1 | Yes | Yes |

[a] Data from Wu and Morris (10).

urease, both of which were found to be present in *Pseudomonas* XA. Column chromatography separated these enzymes from arginine decarboxylase.

Preliminary results have indicated that partially purified arginine decarboxylase from *Pseudomonas* XA, when injected intraperitoneally into mice, is effective in lowering plasma arginine. These results must be confirmed and extended, especially since reports have appeared suggesting that certain neoplasms might regress if exogenous arginine became depleted by administration of an appropriate arginine-degrading enzyme (5, 7, 9).

## ACKNOWLEDGMENTS

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