demonstrate that eq 3a is an adequate expression of the kinetic data, at least at high substrate concentrations.

The steric effect observed in the disproportionation of α-ketoaldehydes with sterically crowded side chains suggests an interesting relationship between binding and catalysis, namely, that good substrates as measured by V_{max} also bind well, but poor substrates as measured by V_{max} bind poorly. The V_{max} for most α-ketoaldehydes studied to date fall in a 3–4-fold range (Vander Jagt et al. 1972a). These substrates show K_M values either comparable to that of methylglyoxal or somewhat smaller if the side chain is apolar. On this basis, one might expect *tert*-butylglyoxal and 2,4,6-trimethylphenylglyoxal to show K_M values lower than that of methylglyoxal and comparable to that of 2,4-dimethylphenylglyoxal. The K_M for *tert*-butylglyoxal is similar to that of methylglyoxal; however, $K_M = K_S$ for *tert*-butylglyoxal but not for methylglyoxal (Vander Jagt and Han, 1973). Since $K_M \geq K_S$, the K_M for *tert*-butylglyoxal, although not directly related to K_M for methylglyoxal, does allow one to conclude that the binding of *tert*-butylglyoxal is not as favorable as one might have expected for this apolar substrate. For 2,4,6-trimethylphenylglyoxal, this relationship between binding and catalysis is even more pronounced. This α-ketoaldehyde is disproportionated by glyoxalase I so slowly that meaningful kinetic parameters could not be obtained. It also binds very poorly based upon the observation that it is not a good competitive inhibitor of the other α-ketoaldehydes. This steric effect is not an inherent property of the substrate, based upon studies of the model reaction (eq 6).

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Biodegradative Ornithine Decarboxylase of *Escherichia coli.* Purification, Properties, and Pyridoxal 5′-Phosphate Binding Site†

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ABSTRACT: The biodegradative ornithine decarboxylase of *Escherichia coli* has been purified to apparent homogeneity. At its pH optimum (pH 7.0), the enzyme exists as a dimer of 160,000 molecular weight. Aggregation of the dimer was promoted by lower pH values. The enzyme requires pyridoxal 5′-phosphate for activity. The coenzyme appears to be bound in Schiff base linkage as suggested by spectral studies and inhibition by $NaBH_4$. The following sequence was determined for the coenzyme binding site: Val-His-(ε-Pxy)Lys-Gln-Gln-Ala-Gly-Gln. The properties of this enzyme are compared with the other biodegradative amino acid decarboxylases that have been isolated from *E. coli.*

Escherichia coli produces two distinct classes of L-amino acid decarboxylases which act, respectively, in biodegradative and biosynthetic roles (reviewed in Morris and Fillingame, 1974). The *biodegradative* decarboxylases are induced by growth at low pH in culture media enriched with amino acids (Gale, 1940). Production of the biodegradative decarboxylases seems to be a defense mechanism against low environmental pH. Bacterial mutants lacking the biodegradative arginine and histidine decarboxylases are unable to grow under acidic conditions (Becker, 1967; Recsei and Snell, 1972; Morris and Fillingame, 1974). The *biosynthetic* ornithine and arginine decarboxylases of *E. coli* are responsible for the synthesis of putrescine and, ultimately,

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spermidine, during growth in neutral minimal medium (Morris and Pardee, 1966; Morris and Koffron, 1969; Morris et al., 1970).

The presence of two different arginine decarboxylases in *E. coli* has been confirmed with purified preparations of both the biodegradative (Blethen et al., 1968; Boeker and Snell, 1968; Boeker et al., 1969, 1971) and the biosynthetic (Wu and Morris, 1973a,b) enzymes. The two arginine decarboxylases differ in pH optimum, metal ion requirements, substrate affinity, subunit molecular weight, and quaternary structure. Antibodies against purified biodegradative arginine decarboxylase do not cross react with, or inhibit the activity of, biosynthetic arginine decarboxylase. Genetic studies show that the gene for the biodegradative enzyme maps between *met B* and *leu* on the *E. coli* chromosome, while that for the biosynthetic enzyme is found close to *ser A* (Maas et al., 1970).

Until the present work, neither the biosynthetic nor the biodegradative ornithine decarboxylase from *E. coli* had been purified and characterized. As a first step in assessing the relationship between the two ornithine decarboxylases, this paper reports the preparation of the biodegradative enzyme in homogeneous form. The properties of this enzyme are compared with the other biodegradative amino acid decarboxylases of *E. coli*. The ornithine decarboxylase has many similarities to the arginine and lysine decarboxylases, suggesting a common evolutionary ancestor.

Materials and Methods

Materials. E. coli UW 44 (ATCC 27549), obtained from the University of Washington clinical microbiology laboratory, produces the biodegradative ornithine, arginine, and lysine decarboxylases and the biosynthetic ornithine and arginine decarboxylases (Applebaum, 1972).

L-Ornithine was obtained from Ajinomoto; pyridoxal 5′-phosphate was from General Biochemicals; bovine liver catalase, horseradish peroxidase, bovine heart lactic dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase were from Worthington Biochemical Corp. Purified rabbit muscle phosphorylase (Sevilla and Fischer, 1969) and biodegradative lysine decarboxylase (Sabo et al., 1974) were isolated by published procedures. All other reagents were of the highest grade available and were used without further purification.

The activity assay for ornithine decarboxylase measured the release of $^{14}CO_2$ from L-[1-^{14}C]ornithine at 37°. The reaction mixture (0.3 ml) contained 100 m*M* Hepes[1] (pH 7.25), 0.04 m*M* pyridoxal phosphate, 1.67 m*M* dithiothreitol, and 7.4 m*M* L-[1-^{14}C]ornithine of appropriate specific activity and enzyme. The released $^{14}CO_2$ was trapped with ethanolamine–methyl Cellosolve (1:2, v/v) and counted as previously described by Morris et al. (1970). The release of CO_2 was stoichiometric with putrescine production (Applebaum, 1972). One unit of ornithine decarboxylase was defined as the amount of enzyme catalyzing the release of 1 μmol of CO_2/min under the above conditions. The specific enzymatic activity was defined as activity units per mg of protein.

Protein Estimation. Protein concentration was determined by the method of Lowry et al. (1951) with crystalline bovine serum albumin as a standard. Correction for β-mercaptoethanol or dithiothreitol was performed as recommended by Vallejo and Lagunas (1970). For calculation of absorbance indices, protein concentrations were determined refractometrically in an analytical ultracentrifuge by the procedure of Babul and Stellwagen (1969), assuming a refractive increment for protein of 0.00185 (Perlmann and Longsworth, 1948).

Polyacrylamide gel electrophoresis with 7 or 7.5% acrylamide gels was performed at pH 8.9 (Ornstein, 1964; Davis, 1964), pH 7.6 (Williams and Reisfield, 1964), and pH 6.6 (Taber and Sherman, 1964) at 4°. The stacking gel was eliminated with no apparent alteration in the banding pattern. Protein solutions containing 10% glycerol were layered above a 10-μl thioglycolate layer (0.1 *M* in 50% sucrose) onto gels which had been prerun for 1 hr (1 mA/tube).

To measure ornithine decarboxylase activity following electrophoresis, gels were run at pH 7.6 and sliced at 1-mm intervals with a Gilson gel slicer. Each gel fraction was washed into a small vial containing 0.25 ml of 0.05 *M* potassium phosphate buffer (pH 6.7) (0.4 m*M* pyridoxal phosphate–10 m*M* β-mercaptoethanol) and assayed in the usual way.

For estimation of subunit molecular weights electrophoresis was performed in 5% acrylamide gel in the presence of 0.1% sodium dodecyl sulfate (Weber and Osborn, 1969). Before electrophoresis, samples were heated at 100° for 3–7 min in 0.01 *M* sodium phosphate buffer (pH 7.2) containing 1% sodium dodecyl sulfate, 1% β-mercaptoethanol, 10% glycerol, and 0.002% Bromophenol Blue. The migration of the protein bands relative to the dye marker was compared to standards of glyceraldehyde-3-phosphate dehydrogenase, phosphorylase *b*, lysine decarboxylase, and ovalbumin.

Sucrose gradient centrifugation was performed by the method of Martin and Ames (1961). After centrifugation, the contents of the tubes were removed with a Buchler peristaltic pump. Peroxidase, lactic dehydrogenase, and catalase were used as markers and assayed as described by Worthington (1968).

Sedimentation velocity experiments were performed at 48,000 rpm at 20–25° with an AN-F or AN-D rotor in aluminum-filled Epon, 2°, double sector cells with standard 12-mm light path. Schlieren patterns were photographed on Kodak Metallographic plates and sedimentation coefficients were calculated as discussed by Schachman (1959). For correction to standard conditions, a partial specific volume of 0.731 calculated from the amino acid analysis (Applebaum, 1972) was used (Cohn and Edsall, 1943). The densities of the potassium phosphate buffers were calculated from International Critical Tables. An Ostwald viscometer equipped with an automatic timing device was used to determine relative viscosities (Autoviscometer, Model 5901A).

Sedimentation equilibrium experiments were performed by the meniscus depletion method of Yphantis (1964). Using ultracentrifuge cells with a multiple channel, 3-mm column centerpiece, protein solutions (0.13 ml) at 1.00, 0.67, and 0.33 mg/ml were placed into the sample channels and 0.13 ml of the corresponding buffer in the buffer channels. The speed of the centrifuge was chosen so that the protein concentration near the meniscus was zero at equilibrium (after 24 hr). The interference patterns were photographed on spectroscopic plates, Type IIG. The fringe displacements along the *X* axis were measured with the Nikon microcomparator. A partial specific volume of 0.731 ml/mg

[1] Abbreviation used is: Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); bicine, *N*,*N*′-bis(2-hydroxyethyl)glycine.

was used in the computations (Applebaum, 1972). The data were processed using the computer program developed by Teller et al. (1969).

Preparation of Apo-ornithine Decarboxylase. Bound pyridoxal phosphate was removed from ornithine decarboxylase by dialysis against 0.25 *M* potassium phosphate buffer (pH 7.0) containing 0.05 *M* L-cysteine, 1 m*M* Na_2EDTA, and 1 m*M* dithiothreitol, similar to the method described by Whanger et al. (1968). Apo-ornithine decarboxylase then was exhaustively dialyzed three times against 500 volumes of 0.05 *M* potassium phosphate buffer (pH 6.7) containing 5 m*M* β-mercaptoethanol. Absorption spectra were measured with a Cary Model 15 recording spectrophotometer.

Isolation and Sequence of the Pyridoxyl Peptide. Approximately 650 mg of protein, estimated to be 80–90% pure by disc and sodium dodecyl sulfate gel electrophoresis, in 170 ml of 0.05 *M* potassium phosphate (pH 6.7) containing 0.4 m*M* pyridoxal phosphate and 10 m*M* β-mercaptoethanol, was reduced with $NaBH_4$ with greater than 95% inactivation. Following denaturation by 8 *M* urea and dialysis against water, the pH was adjusted to 8.0 with NaOH. Thermolysin (12 mg) in 20 ml of 1 m*M* calcium acetate was added and the pH maintained at 8.0 by addition of 0.5 *M* NaOH over a 360-min period. Purification of the phosphopyridoxyllysine-containing material was carried out by the "differential" procedure of Strausbauch and Fischer (1970a,b) as described for lysine decarboxylase (Sabo and Fischer, 1974).

Subtractive Edman degradation was performed as previously described (Sabo and Fischer, 1974). In order to minimize cyclization of glutaminyl residues during Edman degradation, the following modifications were made: (a) coupling with phenyl isothiocyanate was carried out at high pH by making the incubation mixture 3% in triethylamine; (b) all drying steps were carried out on a flash evaporator rather than in a vacuum oven; (c) cyclization and cleavage in trifluoroacetic acid were allowed to proceed for only 5 min; (d) the aqueous peptide solution was not dried down between cycles, but brought to 0.2 ml and a final concentration of 50% pyridine. Aliquots containing approximately 10 nmol were subjected to amino acid analysis on a Beckman Model 120C analyzer. The modified procedure could be carried out much faster, with excellent yields, even on cycles involving glutamine.

Results

Growth of E. coli. Growth experiments and conditions for maximum induction of the enzyme are shown in Table I. The inoculum was grown as described below and the initial cell density in the experimental cultures was approximately 3×10^8/ml. Activity was highest when the bacteria were grown for 6 hr with at least 0.8% ornithine at 30–37° with an initial pH of 5.5. Under optimal conditions, ornithine decarboxylase was induced from an initial specific activity of 0.1–12.8 units/mg. In the final procedure the inoculum (1 l.) was grown in medium 63 of Cohen and Rickenberg (1956), supplemented with trace elements (Ames et al., 1960), 0.2% glucose, and yeast extract (0.05% at 37° with aeration. The inoculation suspension was added to 10 l. of induction medium (1% nutrient broth, 0.05% $(NH_4)_2SO_4$, 0.1% NaCl, 0.1% K_2HPO_4, 0.05% sodium citrate, and 0.8% L-ornithine), adjusted to pH 5.2, and allowed to grow without aeration for 7 hr at 37°. The harvested cells were washed with 0.9% NaCl and used immediately for purification.

Table I: Effect of Growth Conditions on Induction of Ornithine Decarboxylase.[a]

Variable	Range	% Max Sp. Act.
Temp (°C)[b]	22	56
	30	98
	37	100
Temp (+1% glucose, °C)	22	12
	30	22
	37	41
Initial pH[c]	7.0	30
	6.5	49
	6.0	33
	5.5	100
	5.0	87
L-Ornithine·HCl (%)[d]	0.0	1
	0.05	23
	0.1	36
	0.2	58
	0.4	84
	0.6	95
	0.8	98
	1.0	100
Time (hr)	3	57
	6	100
	9	78
	24	88

[a] When one parameter was varied, the others were kept constant; 0.5% ornithine, 0.1% NaCl, 0.1% K_2HPO_4, 0.5% $(NH_4)_2SO_4$, 0.05% sodium citrate, and 1% nutrient broth, pH 5.5, no aeration, 37°. [b] Growth was for 13 hr. [c] Growth was for 7 hr in media containing 0.8% ornithine. [d] Growth was for 7 hr.

Preparation of Crude Extract. The washed cells from the above growth procedure were suspended in 200 ml of starting buffer (0.05 *M* potassium phosphate (pH 6.7)–0.4 m*M* pyridoxal phosphate–10 m*M* β-mercaptoethanol). The cell suspension was cooled with a salt ice bath (−15°) during 20 min of sonication with a Bronson S-75 sonifier at 5.5 A. Cell debris was removed by centrifugation at 35,000*g* for 30 min.

Protamine Sulfate Precipitation. A 2% protamine sulfate solution, prepared at room temperature in starting buffer immediately before use, was added dropwise with stirring (0.025 mg per ml of extract per A_{260}). The A_{260} varied between 43.6 and 48.5 for different preparations. The preparation was stirred for 30 min before centrifugation at 35,000*g* for 30 min. The supernatant solution was slowly adjusted to pH 7.0 with 0.15 *N* NH_4OH.

Ammonium sulfate fractionation was performed with a saturated solution (5°) in 0.05 *M* potassium phosphate buffer (pH 7.0) adjusted to pH 7.0 with concentrated NH_4OH. The protamine sulfate supernatant fluid was brought to 20% saturation with the ammonium sulfate solution and the precipitate was discarded. The supernatant solution was brought to 30% saturation and centrifuged for 20 min at 35,000*g*. The resulting pellet was collected and resuspended in 10 ml of starting buffer.

A typical purification from cells grown in 10 l. of media is summarized in Table II. Ornithine decarboxylase was routinely purified to a specific activity of 125–130 with an average recovery of 85%.

Purity of Ornithine Decarboxylase Preparations. The purified ammonium sulfate fraction showed less than 0.2% decarboxylase activity toward arginine and lysine, indicating no significant contamination by these enzymes. Chro-

Table II: Summary of Purification of Ornithine Decarboxylase from Induced Cells.

Purification Step	Volume (ml)	Total Activity (units)	Total Protein (mg)	Specific Activity (units/mg)	Yield (%)
Crude extract	217	9360	1005	9.3	100
Protamine sulfate (supernatant solution)	221	9060	690	13.1	97
Ammonium sulfate fraction	10.8	8590	66.3	129.6	92

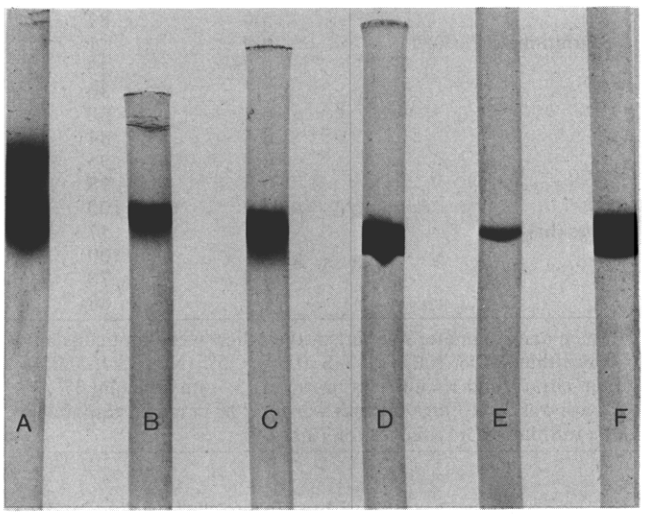

FIGURE 1: Polyacrylamide gel electrophoresis of ornithine decarboxylase: (A) pH 7.6 in the absence of thioglycolate (see Materials and Methods). The following gels were run with thioglycolate: (B) pH 7.6; (C) pH 8.9; (D) pH 6.6. Gels E and F were run in the presence of sodium dodecyl sulfate. Gels A through F contained 22, 12, 8, 13, 1.2, and 19 μg of ornithine decarboxylase, respectively.

matography on agarose 1.5M showed a constant specific activity over most of the activity peak. The results from analysis of the ammonium sulfate fraction by polyacrylamide disc gel electrophoresis appear in Figure 1. Two diffuse bands were seen after electrophoresis at pH 7.6 in the absence of thioglycolate. When thioglycolate was included, one distinct band was seen at pH 7.6, 6.6, or 8.9. In the presence of 0.1% sodium dodecyl sulfate a single band was also seen (Figure 1E and F). Ornithine decarboxylase activity migrated in a single peak corresponding to the material staining as protein (Figure 2).

Enzyme Kinetics. The pH dependence of enzymatic activity was determined with the following buffers: 0.1 *M* sodium acetate, 0.05 *M* Mes, 0.1 *M* Pipes, 0.1 *M* Hepes, 0.1 *M* bicine, and 0.1 *M* glycine-NaOH. A bell-shaped curve, centered at pH 6.9, was obtained. Approximately 50% of the activity remained at pH 6.0 and 7.8.

The dependence of activity on ornithine concentration showed classical Michaelis–Menten kinetics using purified ornithine decarboxylase. When these data were plotted as V vs. V/S and the resulting points fitted by the least-squares method, a K_m of 3.6 m*M* was obtained (Table III). Putrescine and its biosynthetic product spermidine inhibit ornithine decarboxylase. In the presence of either polyamine the apparent K_m was increased and the V_m was decreased (Table III).

Molecular Weight and Subunit Structure. Ornithine decarboxylase was subjected to polyacrylamide electrophoresis in the presence of 0.1% sodium dodecyl sulfate. Seven determinations gave values corresponding to a subunit molecular weight of 80,000 ± 3% when compared to marker proteins (see Materials and Methods). In sedimentation equilibrium experiments performed at pH 6.7 the molecular weight of the enzyme depended somewhat on protein concentration (Figure 3). The molecular weight increased across the cell to a value of 165,000–170,000 near the bottom with the smallest observable species 88,000 ± 3400. (The uncertainty is the weighted root-mean-square value, Teller et al., 1969.) It is concluded that the native enzyme is a dimer of 80,000 subunit molecular weight.

Influence of pH on the Sedimentation Coefficient of Ornithine Decarboxylase. In sedimentation velocity experiments at pH 8.0 and 7.0, ornithine decarboxylase sedimented as a single symmetrical boundary with $s_{20,w}$ equal to 6.4 S and 6.2 S, respectively (0.05 *M* potassium phosphate, protein concentration 4 mg/ml). At pH 6.0, the enzyme showed a pronounced tendency to aggregate (Figure 4), with the fastest sedimenting species having a sedimentation coefficient of 17.2 S. These results were confirmed by sucrose density gradient centrifugation.

Table III: Polyamine Inhibition of Ornithine Decarboxylase.

Polyamine	Concn (m*M*)	K_m or K_m^{app}[a] (m*M*)	V_m[a] (μmol min^{-1} mg of protein^{-1})
None		3.6 ± 0.2	193 ± 4
Putrescine	6	4.3 ± 0.4	145 ± 7
	12	6.0 ± 0.5	125 ± 6
Spermidine	2	5.9 ± 1.2	158 ± 19
	5	10.7 ± 1.1	153 ± 11

[a] Values for K_m and V_m were obtained from plots of V vs. V/S which were fitted by the least-squares method. The error is expressed as ±1 SD.

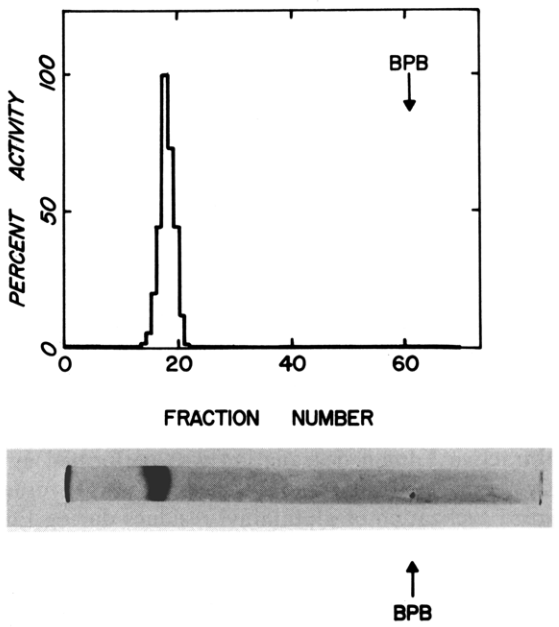

FIGURE 2: Coincidence of ornithine decarboxylase activity and material staining as protein after gel electrophoresis. Ornithine decarboxylase was assayed in gel slices (see Materials and Methods) after electrophoresis of 1.4 μg of purified enzyme at pH 7.6. An identical gel was stained with Coomassie Blue. The position of the Bromophenol Blue marker (BPB) is indicated by the arrow.

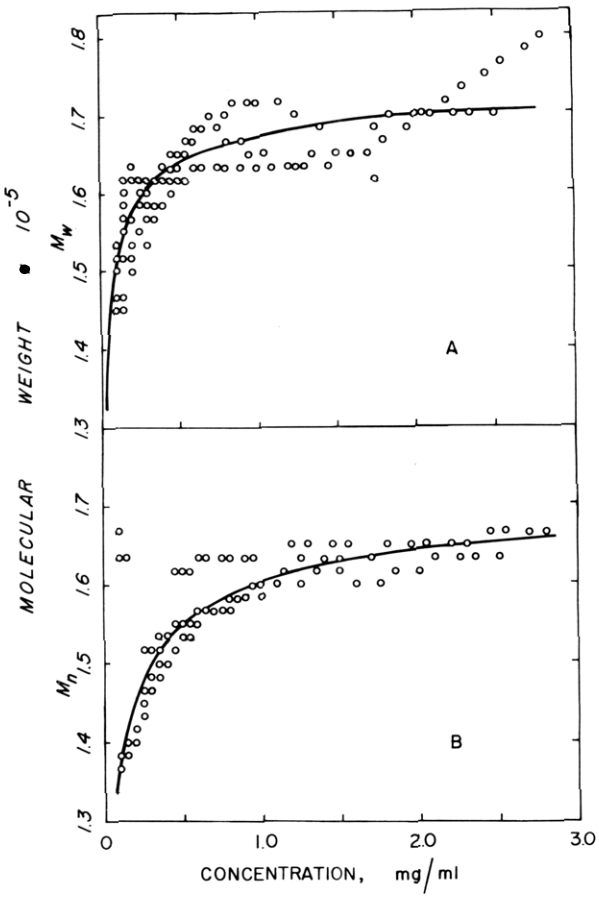

FIGURE 3: Molecular weight distribution of ornithine decarboxylase as a function of protein concentration. The enzyme was dialyzed against 0.05 *M* potassium phosphate buffer (pH 6.7) containing 0.4 m*M* pyridoxal phosphate and 10 m*M* β-mercaptoethanol. Sedimentation equilibrium ultracentrifugation was performed at 12,000 rpm for 24 hr with temperature maintained at 5°. The weight-average (A) and number-average (B) molecular weights were calculated as described under Materials and Methods.

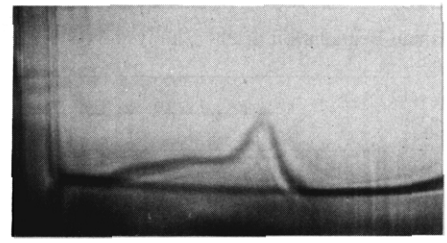

FIGURE 4: Sedimentation of ornithine decarboxylase at pH 6.0. Purified ornithine decarboxylase was dialyzed against 0.05 *M* potassium phosphate buffer (pH 6.0) supplemented with pyridoxal phosphate and β-mercaptoethanol as described under Materials and Methods. Protein concentration was 4 mg/ml. The schlieren pattern was photographed 27.8 min after reaching speed (48,000 rpm) at a bar angle of 55°. Direction of sedimentation is from left to right.

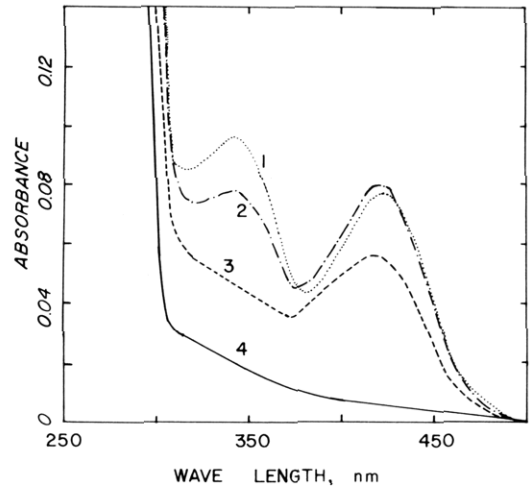

FIGURE 5: Absorption spectra of ornithine decarboxylase: holoenzyme (1.3 mg/ml) at pH 6.0 (curve 1); holoenzyme (1.4 mg/ml) at pH 7.1 (curve 2); holoenzyme (1.4 mg/ml) at pH 8.1 (curve 3); and apoenzyme (0.7 mg/ml) at pH 6.7 (curve 4). All spectra were recorded on a Cary Model 15 spectrophotometer, using the expanded scale.

Pyridoxal Phosphate Binding. Studies with apoenzyme were performed immediately following preparation. Under these conditions activity could be completely restored by readdition of pyridoxal phosphate. After 2–3 days of storage, only 66% of the holoenzyme activity could be reconstituted. Apoenzyme, assayed in the absence of pyridoxal phosphate, contained less than 3% of the holoenzyme activity.

The absorption maximum seen with apoenzyme was at 280 nm, characteristic of aromatic amino acid residues, with a small amount of absorption between 310 and 390 nm (Figure 5, curve 4). The spectrum of holoornithine decarboxylase, investigated as a function of pH, is shown in Figure 5. At pH 6.0 and 7.1, the spectra showed maxima at 420 and 340 nm. At pH 8.1, the total absorbance between 340 and 420 nm decreased. Between 310 and 370 nm, the spectrum at pH 8.1 resembled that of the apoenzyme. A shoulder on the long wavelength band appeared at approximately 390 nm which is characteristic of free pyridoxal phosphate. These results suggest a weakened binding of pyridoxal phosphate at higher pH values.

Sequence of the Phosphopyridoxyl Peptide. The purification of the phosphopyridoxyllysine-containing material after thermolysin digestion was carried out as described under Materials and Methods. The amino acid composition and subtractive Edman analysis (Table IV) revealed that a mixture of two overlapping peptides of identical charge had been isolated. Nearly 2 equiv of pyridoxyllysine were present relative to glycine and alanine and sequential degradations removed approximately 2 equiv of a single amino acid on cycles one through five. On turns six and seven, the stoichiometry dropped abruptly to approximately one. These findings suggested penta- and octapeptides, present in approximately equal amounts. The amino termini were in register, consistent with a partial cleavage by thermolysin at alanine. The carboxyl terminus of the longer peptide was assigned as a glutamyl residue on the basis of the initial amino acid composition and from the compositions following the sixth and seventh cycles.

All of the glutamyl residues arose from the γ amides, as shown by carboxypeptidase digestion (1:50 molar ratio, 4 hr, 37°, pH 8.0). Since no threonine or serine was present, glutamine could be quantitated by amino acid analysis and a norleucine standard was used to calculate recoveries. No free glutamic acid, which is released rapidly under these conditions, could be detected. Since carboxypeptidase digestion was blocked at pyridoxyllysine only glutamine, glycine, and alanine were released (3.24, 0.58, and 0.55 equiv, respectively).

Discussion

Four biodegradative amino acid decarboxylases from *E. coli* have now been purified and characterized (Boeker and

Table IV: Edman Degradation of Phosphopyridoxyl Peptide.

Residue	Composition after Cycle Number							
	0	1	2	3	4	5	6	7
(ε-Pxy)lysine	1.77	1.77	2.05	0.00				
Histidine	2.10	2.55	0.68	0.00				
Glutamic acid	5.23	4.87	4.96	4.82	2.95	1.29	1.19	1.00
Glycine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.59
Alanine	0.97	0.85	0.85	0.85	0.86	0.98	0.38	0.23
Valine	2.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Residue(s) lost		Val Val	His	(ε-Pxy)Lys (ε-Pxy)Lys	Glx Glx	Glx Glx	Ala	Gly

Table V: Properties of the Biodegradative Amino Acid Decarboxylases.

Substrate	Optimum pH	Turnover No.[a]	K_m[b]	Subunit Mol Wt × 10^{-3}	No. of Subunits	Ref
Ornithine	7.0	10.4	3.6	80	2	
Arginine	5.2	32.8	0.65	82	2 or 10	*c*
Lysine	5.7	80	1.5	80	2 or 10	*d*
Glutamate	3.8	5.5	0.54	50	6	*e*

[a]nmol per min per subunit. [b]m*M*. [c]Blethen et al. (1968); Boeker and Snell (1968); Boeker et al. (1969). [d]Sabo et al. (1974). [e]Strausbauch and Fischer (1970a,b).

Snell, 1972); these having glutamic acid, arginine, lysine, and ornithine as substrates. The four enzymes all require pyridoxal phosphate for activity and, at their respective pH optima, appear to bind pyridoxal phosphate in a Schiff base linkage as evidenced from their absorption spectra. Some of the other properties of the biodegradative amino acid decarboxylases are summarized in Table V. All have pH optima at neutrality or lower, with ornithine decarboxylase being the highest at pH 7.0. They differ widely in the catalytic parameters of turnover number and K_m. Glutamic acid decarboxylase, as a hexamer of 50,000 subunit molecular weight, is exceptional in its quaternary structure. The three basic amino acid decarboxylases show definite structural similarities. All three have a subunit molecular weight of approximately 80,000 and the fundamental unit of the native enzymes appears to be a dimer of 160,000 molecular weight. At neutral pH and moderately high ionic strength, the dimeric units of the arginine and lysine decarboxylases aggregate to decamers, dimers being stable only at high pH and low ionic strength. Under conditions which favor formation of the decamer of the lysine or arginine enzymes, ornithine decarboxylase still exists as a dimer. Aggregation of the ornithine decarboxylase dimer can be induced by lowering the pH to 6.0. Therefore, although the fundamental unit of all three enzymes is a dimer and aggregation in all cases is favored by low pH, the dimer of ornithine decarboxylase shows much less tendency toward association than was seen with the other two enzymes.

The amino acid sequences about the ε-pyridoxyllysine residues of these four biodegradative decarboxylases have been determined (Table VI). The strong homology between the arginine and lysine decarboxylases was noted previously (Sabo and Fischer, 1974). All four decarboxylases possess a histidyl residue to the amino side of the phosphopyridoxyllysine. It was previously suggested that the imidazole group of this invariant residue might either participate directly in the catalytic process or form an ion pair with the phosphate residue of the coenzyme (Strausbauch and Fischer, 1970b). The three basic amino acid decarboxylases have an extremely hydrophobic region to the carboxyl side of the coenzyme binding site which might form part of a binding pocket for the coenzyme (Sabo and Fischer, 1974). Aside from these general features of the histidyl residue and the nonpolar sequence, the peptide from ornithine decarboxylase shows none of the strong homology in actual amino acid sequence that was seen between the arginine and lysine decarboxylases.

With the evidence now available, one can consider the evolution of the biodegradative amino acid decarboxylases of *E. coli*. There is no compelling reason to argue that glutamic acid decarboxylase shares a common ancestor with the basic amino acid decarboxylases. Glutamic acid decarboxylase differs from the others in nearly all of the structural features outlined above. On the other hand, all three basic amino acid decarboxylases are structurally quite similar. Of special note are the identical subunit molecular weights and the dimeric quaternary structure. If these three enzymes arose from a common ancestor, one would argue on the basis of the amino acid sequences of the phosphopyridoxyllysine-containing peptides that the lysine and arginine decarboxylases diverged much more recently than did the ornithine decarboxylase.

Table VI: Pyridoxal 5′-Phosphate Binding Sites of the Biodegradative Decarboxylases of *E. coli*.

Substrate	Sequence	Ref
Ornithine	Val-His-(ε-Pxy)Lys-Gln-Gln-Ala-Gly-Gln	
Lysine	. . .Tyr-Glu-Thr-Glu-Ser-Thr-His-(ε-Pxy)Lys-Leu-Leu-Ala-Ala-Phe	*a*
Arginine	Ala-Thr-His-Ser-Thr-His-(ε-Pxy)Lys-Leu-Leu-Asn-Ala-Leu. . .	*b*
Glutamic acid	Ser-Ile -Ser -Ala-Ser-Gly-His-(ε-Pxy)Lys-Phe	*c*

[a]Sabo and Fischer (1974). [b]Boeker et al. (1971). [c]Strausbauch and Fischer (1970b).

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