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Chemical Properties of *Escherichia coli* Lysine Decarboxylase Including a Segment of Its Pyridoxal 5′-Phosphate Binding Site†

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ABSTRACT: The inducible lysine decarboxylase of *Escherichia coli* has been further characterized in terms of its chemical properties, including amino acid composition. The enzyme contains six sulfhydryl groups which react very slowly if at all in the native decamer; one SH group per monomer becomes highly reactive upon dissociation of the enzyme to the dimer. No immunological cross-reactivity could be demonstrated among the inducible *Escherichia coli* lysine, arginine, ornithine, and glutamic acid decarboxylases. The following sequence was determined for the cofactor binding site: Val-Ile-Tyr-Glu-Thr-Glu-Ser-Thr-His-(ε-Pxy)Lys-Leu-Leu-Ala-Ala-Phe. Sequence homologies of the active site of various decarboxylases suggest that certain residues (*e.g.*, a histidyl residue adjacent to ε-phosphopyridoxyllysine) may play a direct role in catalysis. Further homologies in the sequence of the amino-terminal regions of lysine and arginine decarboxylases support the proposition that these enzymes are structurally related, probably as a result of divergent evolution.

We have shown (Sabo *et al.*, 1974) that lysine decarboxylase from *Escherichia coli* is an inducible enzyme with a subunit molecular weight of 80,000. Hydrodynamic studies and electron microscopic data demonstrated that it can exist as a dimer (7.8 S, mol wt 158,000), a decamer (22.2 S, mol wt 780,000), or as higher aggregates. Although the inducible *E. coli* glutamic acid decarboxylase has very different chemical and physical properties, the striking similarities in subunit size and quaternary structure between lysine and arginine decarboxylases suggested that these two enzymes might be structurally related. These comparative studies have now been extended to include the sequence of the pyridoxal-P binding site of lysine decarboxylase.

Since the finding that pyridoxal-P could be irreversibly fixed to glycogen phosphorylase by reduction of the aldimine linkage with $NaBH_4$ (Fischer *et al.*, 1958; Strausbauch *et al.*, 1967b; Forrey *et al.*, 1971b), the technique has been applied to a variety of pyridoxal-P containing enzymes, including the mitochondrial and extramitochondrial aspartate aminotransferases from pig heart (Morino and Watanabe, 1969), inducible glutamic acid and arginine decarboxylases from *E. coli* (Strausbauch and Fischer, 1970b; Boeker *et al.*, 1971), tryptophanase and tryptophan synthetase (Kagamiyama *et al.*, 1970; Fluri *et al.*, 1971; Mauer and Crawford, 1971), and pyridoxamine–pyruvate transaminase (see Snell and DiMari, 1970). The separation of phosphopyridoxyl peptides has been considerably simplified by the introduction of a "differential" procedure in which phosphorylated derivatives are chromatographed before and after alkaline phosphatase treatment (Strausbauch and Fischer, 1970b).

Sequence determination of the pyridoxal-P binding site in glutamic acid and arginine decarboxylases indicated that a histidyl residue occurs adjacent to the phosphopyridoxyllysine group. Given the distinct catalytic properties of imidazole side chains and their relative scarcity in proteins, it was suggested that this residue could be functionally important in decarboxylation (Strausbauch and Fischer, 1970b; Boeker *et al.*, 1971). However, since data were available for only two enzymes, this juxtaposition could have been purely fortuitous. We have therefore extended this analysis to the inducible *E. coli* lysine decarboxylase, have described its

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Dedicated to Professor A. E. Braunstein for his 70th Birthday.

purification and certain physical properties (Sabo *et al.*, 1974), and report here some of its chemical properties, including amino acid analysis, reactivity of SH groups, sequence of a segment of the pyridoxal-P binding site and some immunological studies.

Materials and Methods

Lysine decarboxylase was purified to homogeneity from *E. coli* B and assayed as described in the preceding publication (Sabo *et al.*, 1974); the apodecarboxylase was also prepared as previously described. Sodium borohydride reduction was carried out according to Fischer *et al.* (1958) and Strausbauch *et al.* (1967b). A 200-fold molar excess of $NaBH_4$ was dissolved in cold distilled water to give a 0.02 M solution which was added immediately to the enzyme at neutral pH. After 10 min, the solution was spun at 16,000*g* for 15 min to break up the foam. Enzymatic activity is quantitatively destroyed by this procedure.

Arginine and glutamic acid decarboxylases were prepared according to Boeker *et al.* (1969) and Strausbauch *et al.* (1967a), respectively. Inducible *E. coli* ornithine decarboxylase and the homologous antiserum were gifts of Dr. D. Applebaum.

Thermolysin was obtained from Calbiochem, alkaline phosphatase, diisopropyl fluorophosphate treated carboxypeptidases A and B and L-1-tosylamido-2-phenylethyl chloromethyl ketone treated trypsin from Worthington, and nagarse from the Biddle Sawyer Corp., New York, N. Y. Dowex 1 and 50 (Bio-Rad) were prepared for use according to Schroeder (1967a,b). *N*-Ethylmorpholine and α-picoline were redistilled. The phenyl isothiocyanate, pyridine, and ethyl acetate used for sequence analysis were Sequanal grade from Pierce Chemical Co., Rockford, Ill. Urea was passed through a mixed-bed ion-exchange column and recrystallized from water and ethanol. Anhydrous hydrazine was prepared by distillation of 95% hydrazine (Eastman) under reduced pressure.

Pyridoxal 5'-phosphate was determined by the phenylhydrazine procedure of Wada and Snell (1961). Solutions containing 1–3 mg of protein were dialyzed exhaustively against potassium phosphate buffer, pH 8.0, μ 0.10, and deproteinized with an equal volume of 0.6 N perchloric acid. To measure the total potential pyridoxal-P binding sites, enzyme solutions were incubated at 4° overnight with a threefold molar excess of the cofactor and chromatographed on a 1 × 30 cm column of Sephadex G-25 equilibrated with 0.05 M sodium succinate (pH 6.0). Fresh solutions of pyridoxal-P were used as standards; their concentration was determined spectrophotometrically using a molar absorbance of 6600 at 388 nm in 0.1 M NaOH (Pederson and Sober, 1954).

For reconstitution studies, lysine apodecarboxylase was incubated for 1 hr at 4° with varying amounts of pyridoxal-P. The extent of reactivation was determined by the standard assay except that pyridoxal-P was omitted.

Amino Acid Analysis. Lysine decarboxylase was dialyzed exhaustively against 0.1 M KCl followed by distilled water. Duplicate lyophilized samples of 1 mg of enzyme and 0.1 μmol of norleucine added as an internal standard (Walsh and Brown, 1962) were hydrolyzed at 110° for 24, 48, 72, and 96 hr in 5.7 N HCl in sealed evacuated tubes which had been repeatedly flushed with N_2. Analyses were performed on a Beckman Model 120C amino acid analyzer according to Spackman *et al.* (1958) and Spackman (1963).

The same system was used for analysis of pyridoxyl peptides (following 24-hr hydrolysis) except that basic residues were determined on a 20-cm column of Aminex Q-15S which separates lysine from N^{ϵ}-pyridoxyllysine (Forrey *et al.*, 1971a). Since none of the peptides described below contained lysine, this column was used only for initial compositions.

The cysteine plus cystine content was determined on duplicate samples after performic acid oxidation of the protein and 24-hr hydrolysis according to the procedure of Moore (1963) as modified by Hirs (1967a). Determination of free sulfhydryl groups was carried out by titration with 1 mM 5,5'-dithiobis(2-nitro-benzoic acid) (Nbs_2)[1] in 8 M urea according to Ellman (1959). Duplicate tryptophan determinations were made spectrophotometrically according to Bencze and Schmid (1957).

Antisera were prepared by intradermal injection of 1 ml of an emulsion of equal volumes of complete Freund's adjuvant and 8 mg/ml of decarboxylase in 0.15 M NaCl above each leg of two 7–10-lb female New Zealand rabbits. Four weeks later 50 ml of blood was taken from the ear vein and the animals were again injected with antigen. Bleeding was repeated after an additional 2 weeks. Serum was prepared from whole blood and stored frozen.

Paper chromatography and electrophoresis was carried out on Whatman 3MM paper. Chromatography was run in 1-butanol–pyridine–acetic acid–water (90:60:18:72, Bennett, 1967) with a Bromophenol Red marker. Electrophoresis was carried out at pH 3.6 (2500 V) in pyridine–acetic acid–water (1:10:389, Michl, 1951) and at pH 6.5 (2000 V) in pyridine–acetic acid–water (286:1:2570).

Isolation of Pyridoxyl Peptides. Two overlapping pyridoxyl peptides were isolated by the "differential" procedure of Strausbauch and Fischer (1970b) following (a) thermolysin or (b) chymotrypsin digestion of the denatured (8 M urea) $NaBH_4$-reduced enzyme. Following exhaustive dialysis against distilled water the solutions were adjusted to pH 8.0 with NaOH, and maintained at this value throughout the proteolysis by addition of 0.5 M NaOH. For (a), an 8-ml aliquot of a 1-mg/ml solution of thermolysin in 1 mM calcium acetate was added to 800 mg (10 μmol) of protein suspension; digestion was allowed to proceed for 5 hr at 37°. For (b), a solution containing 10 mg of α-chymotrypsin was added to 1 g (12.5 μmol) of protein and digestion was carried out at room temperature for 160 min.

Lyophilized digests were dissolved in 98% formic acid, diluted with six volumes of distilled water, and applied to a 1 × 100 cm column of Dowex 50W-X2, 200–400 mesh, which was developed with a pyridine–acetic acid gradient from pH 3.1 to 5.0 at 38° according to Schroeder (1967a). Fractions were analyzed for fluorescence at 390 nm in a Farrand spectrofluorimeter and for peptide material with ninhydrin after alkaline hydrolysis (Hirs, 1967b).

Pooled lyophilized fluorescent fractions were chromatographed on a 0.6 × 60 cm column of Dowex 1-X2, 200–400 mesh, which was developed at 38° according to Schroeder (1967b) except that the pH 9.4 buffer was omitted from the gradient for digest (a) while an additional buffer at pH 7.4 was introduced in the gradients for both (a) and (b).

Final purification was achieved by the "differential" approach of Strausbauch and Fischer (1970b). Peptide mixtures were incubated with 50 μg of *E. coli* alkaline phosphatase for 24 hr at 30°, to hydrolyze the 5'-phosphate ester of the phosphopyridoxyl peptide. The removal of two negative charges from the peptides selectively altered their chromatographic

[1] Abbreviation used is: Nbs_2, 5,5'-dithiobis(2-nitrobenzoic acid).

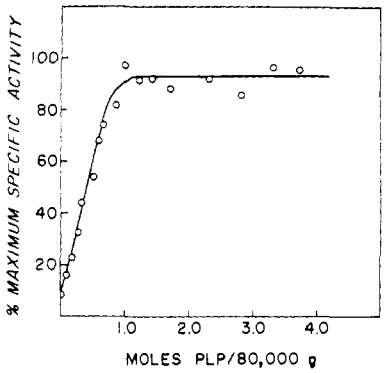

FIGURE 1: Reactivation of lysine apodecarboxylase by pyridoxal-5'-P. The enzyme was incubated at room temperature for 10 min with varying amounts of pyridoxal-P. The maximum specific activity (900 μmol of CO_2 released min^{-1} mg^{-1}) refers to the preparation before resolution.

behavior while contaminating peptides remained unchanged. When the digests were then rechromatographed on Dowex 1 under precisely the same conditions, the fluorescent peptides eluted earlier in the gradient, well ahead of contaminating material; these fractions were lyophilized and redissolved in distilled water.

Peptide Sequence Determination. Subtractive Edman degradations were carried out according to Gray (1967) except that amino acids were identified subtractively by analysis rather than as their dimethylaminonaphthalenesulfonyl derivatives. Carboxypeptidase A (1:25 molar ratio at pH 8.0) and B (1:50 molar ratio at pH 7.0) digestions were carried out for 4 hr on 40 nmol of material in 0.2 M *N*-ethylmorpholine acetate buffer. Hydrazinolysis was performed according to Braun and Schroeder (1967); *ca.* 20 nmol of material, including 0.1 μmol of norleucine, was incubated in a sealed evacuated tube for 80 hr at 80° with 2 ml of anhydrous hydrazine in the presence of 50 mg of Amberlite CG-50. The mixture was filtered, dried over H_2SO_4, and applied to the amino acid analyzer.

Amino-terminal sequences of lysine and arginine decarboxylase were determined with a Beckman Model 890A automatic protein sequencer as described by Edman and Begg (1967) following carboxymethylation of the lysine with [^{14}C]-iodoacetic acid (5×10^4 dpm/μmole).[2]

Results

Pyridoxal 5'-Phosphate Binding. To saturate the enzyme and increase its heat stability, an excess of cofactor is added to the crude extract; however, free or weakly bound pyridoxal-P is removed during purification which includes two precipitation steps and exhaustive dialysis (Sabo *et al.*, 1974). The final product was found to contain 0.88 ± 0.03 mol per 80,000 g of protein. This represents a nearly complete saturation of sites since treatment with excess cofactor immediately prior to the measurements increases the amount bound only slightly, to 1.05 ± 0.04. Both values are the average of four determinations.

When the apoenzyme (8% residual activity) was reconstituted with varying molar ratios of cofactor, more than 90% reactivation was achieved. Maximum activity occurred at a ratio of 1.0–1.2 mol of pyridoxal-P per 80,000 g of protein as shown in Figure 1. Activity was proportional to the amount of cofactor present below this ratio and remained constant above it.

The *amino acid composition* of the enzyme is reported in Table I. The difference between duplicate samples was less than 3% for each amino acid except for proline and ammonia which differed by 3.5 and 4.5%, respectively.

TABLE I: Amino Acid Composition of Lysine Decarboxylase.[a]

Residue	g/100 g	Mol/80,000 g
Lysine	6.61 ± 0.10	41.3
Histidine	3.69 ± 0.03	21.5
Ammonia	1.14 ± 0.05	53.9
Arginine	5.60 ± 0.14	28.7
Aspartic acid	10.70 ± 0.03	74.5
Threonine	5.92 ± 0.04	46.9
Serine	4.12 ± 0.03	37.9
Glutamic acid	10.52 ± 0.03	65.2
Proline	4.79 ± 0.05	39.5
Glycine	3.07 ± 0.02	43.1
Alanine	4.11 ± 0.01	46.3
Valine	4.60 ± 0.04	37.5
Methionine	4.80 ± 0.03	29.3
Isoleucine	6.58 ± 0.08	46.6
Leucine	10.57 ± 0.07	54.5
Tyrosine	6.49 ± 0.05	31.9
Phenylalanine	6.04 ± 0.07	32.9
Cysteine	0.77 ± 0.00	6.0
Tryptophan	4.17 ± 0.08	9.6

[a] All values except cysteine and tryptophan (see Methods) are averages computed from duplicate determinations following 24-, 48-, 72-, and 96-hr hydrolysis. Ammonia, threonine, and serine were determined by extrapolation to zero time of hydrolysis; valine and isoleucine values are for 96-hr hydrolysis.

Sulfhydryl Content. By performic acid oxidation, 6.0 mol of cysteic acid was found per 80,000 g of protein (Table I). All were apparently derived from the oxidation of cysteinyl (as opposed to cystinyl) residues since determination of free sulfhydryl groups by titration with Nbs_2 in 8 M urea (Figure 2, top) gave a value of 6.2 residues/subunit.

The degree to which these groups are available for reaction in the holo- and apoenzyme in the absence of denaturing agents is also shown in Figure 2. The major conclusions from these data are as follows: (a) sulfhydryl groups of the apoenzyme tend to react more readily than do those of the holoenzyme; (b) in both apo and holo dimers (pH 8.0, μ 0.02), one SH group per subunit reacts preferentially; (c) holo decamers (μ 0.10), which react extremely slowly, if at all, at pH 7.0, but are considerably more reactive at pH 8.0, show no evidence of a uniquely reactive SH group.

Immunological Properties. A single precipitin band is formed in agar during diffusion of 5 μl of lysine decarboxylase antiserum against 0.25–50 μg of enzyme. Dimeric and decameric structures, apoenzyme, and $NaBH_4$-reduced lysine decarboxylase all reacted equally well. Heterologous antigens, such as inducible *E. coli* arginine, glutamic acid, and ornithine decarboxylases, displayed no cross-reactivity (Figure 3). In addition, antisera to arginine, glutamic acid, and ornithine

[2] Detailed sequence data were submitted to the reviewers and are available from the authors upon request.

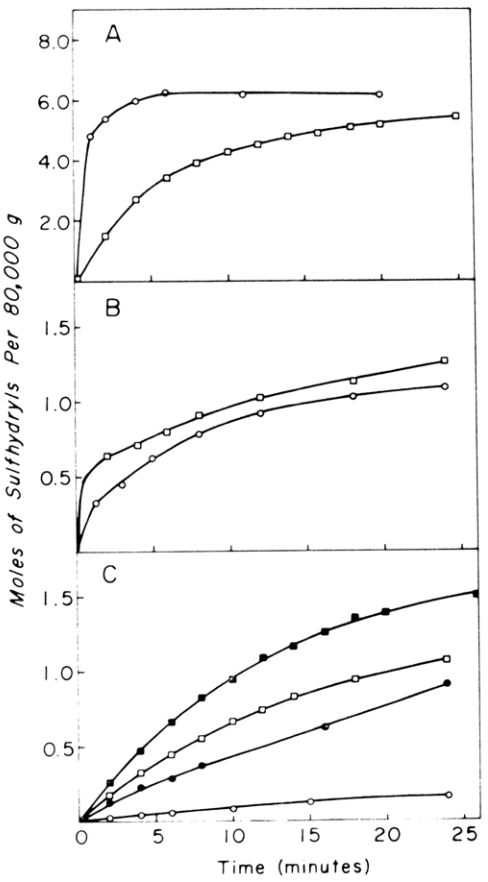

FIGURE 2: Reaction of lysine decarboxylase with 1 mM Nbs_2. (A) Determination of total sulfhydryl groups in 8 M urea (○) and 1% sodium dodecyl sulfate (□). Protein concentration was 8 mg/ml. (B) Titration of enzyme dimer (3 mg/ml) in potassium phosphate (pH 8.0), μ 0.02: apoenzyme (□) and holoenzyme (○). (C) Reaction of decamer (3 mg/ml) in potassium phosphate, μ 0.10: apoenzyme (pH 7.0) (□); apoenzyme, pH 8.0 (■); holoenzyme, pH 7.0 (○); holoenzyme, pH 8.0 (●).

decarboxylases did not cross-react with the heterologous antigens.

Sequence of Phosphopyridoxyl Peptides. The preparation of peptides Th-1 and Ct-1 after thermolysin and chymotrypsin digestion of $NaBH_4$-reduced lysine decarboxylase is described under Methods. Peptide Th-1 was purified from digest (a) twice with overall yields of 30 and 25% (see Figure 4); its amino acid composition (Table II) suggested a decapeptide.

Subfragments of Th-1 with the compositions given in Table II were prepared by digesting *ca.* 0.5 μmol of the peptide in 0.25 ml of 0.1 M ammonium acetate (pH 8.5) with 1 mg of nagarse for 1 hr at 30°, followed by paper electrophoresis at pH 6.5 for 1 hr. Three ninhydrin-positive spots, designated Na-1 to Na-3, were obtained with R_F values of 1.00, −0.32, and −0.73, respectively, relative to Na-1, the most basic peptide. Each was eluted with distilled water.

Fractionation of digest (b) to give peptide Ct-1 (Figure 5) was achieved with an overall yield of only 5%, probably due to photodecomposition of the phosphopyridoxyllysine group during prolonged storage of the reduced enzyme. The amino acid composition shown in Table II indicated that it was a hexapeptide.

Amino acid sequence data were obtained primarily by the subtractive Edman procedure and are summarized in Table III along with the total sequence of the pyridoxal-P binding site deduced from these peptides.

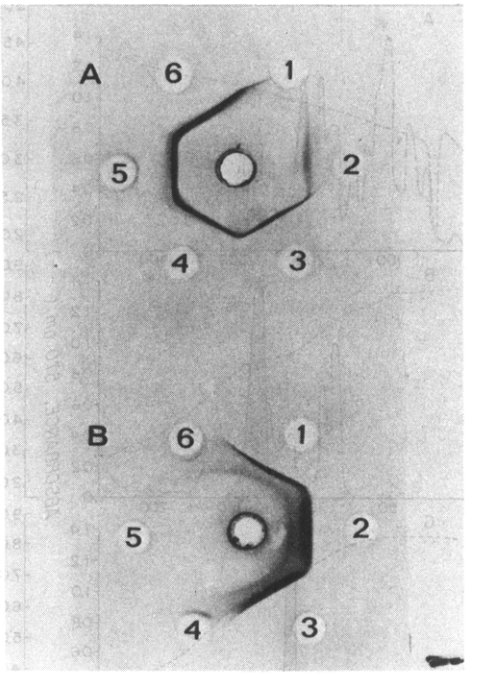

FIGURE 3: Double diffusion of antiserum to lysine decarboxylase *vs.* various antigens. Center wells contained 5 μl of antiserum; precipitin bands were stained with Amido Black. (A) From 1 to 6: 0.25, 1.0, 5.0, 10, 25, and 50 μg of native lysine decarboxylase. (B) 10 μg each of (1) dimeric species, (2) apoenzyme, (3) $NaBH_4$-reduced enzyme, (4) arginine decarboxylase, (5) glutamic acid decarboxylase, and (6) ornithine decarboxylase.

TABLE II: Amino Acid Compositions of Pyridoxyl Peptides.

Amino Acid	Th-1	Na-2	Na-3	Na-1[a]	Ct-1
(ε-Pxy)Lysine	0.77			1.42	0.93
Histidine	1.00			1.69	
Threonine	2.13		0.62	1.00	
Serine	0.90			0.82	
Glutamic acid	2.18	1.00	1.00		
Alanine					2.00
Valine	0.49[b]	0.30			
Isoleucine	0.57[c]	0.40			
Leucine					1.91
Tyrosine	1.06	0.25[d]			
Phenylalanine					1.16

[a] Mixture of di- and tetrapeptide. [b] After 48-hr hydrolysis this value increased to 0.80. [c] After 48-hr hydrolysis this value increased to 0.92. [d] Recoveries of tyrosine following paper electrophoresis were consistently low.

Amino-Terminal Sequences. Methionine was reported previously to be the NH_2-terminal residue of both arginine (Boeker *et al.*, 1969) and glutamic acid decarboxylases (Strausbauch and Fischer, 1970a). The first seven residues in the sequence of lysine and arginine decarboxylases were unambiguously identified in duplicate sequencer runs: lysine decarboxylase: Met-Asn-Val-Ile-Ala-Ile-Leu; arginine decarboxylase: Met-Lys-Val-Leu-Ile-Val-Glu.

Discussion

All known amino acid decarboxylases possess a carbonyl group and an electron sink which act in concert during ca-

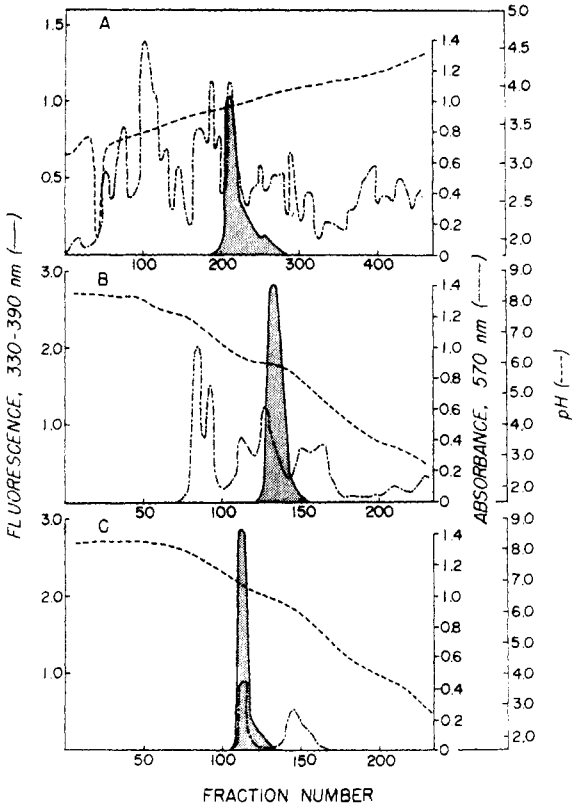

FIGURE 4: Purification of pyridoxyl peptide Th-1 following thermolysin digestion of $NaBH_4$-reduced lysine decarboxylase: (A) ion-exchange chromatography of total digest on Dowex 50; (B) chromatography of fluorescent material on Dowex 1; (C) rechromatography of fluorescent fractions on the same Dowex 1 column after digestion with *E. coli* alkaline phosphatase.

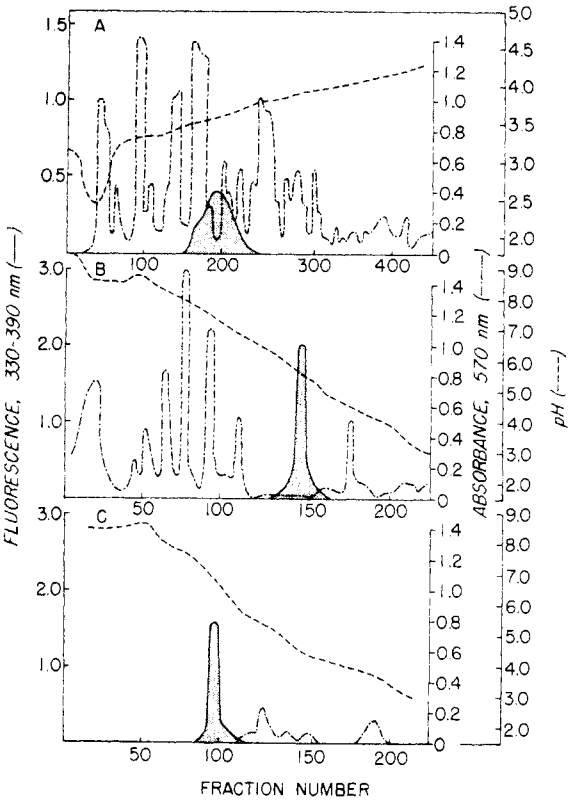

FIGURE 5: Isolation of the pyridoxyl peptide Ct-1 from a chymotryptic digest of $NaBH_4$-reduced lysine decarboxylase: (A) Dowex 50 fractionation of crude digest; (B) Dowex 1 chromatography of the fluorescent fractions obtained above; (C) rechromatography on Dowex 1 following cleavage of the 5′-phosphate ester of the phosphopyridoxyl peptide.

talysis (Snell and DiMari, 1971). Most utilize pyridoxal-5′-P for this purpose. Lysine decarboxylase contains stoichiometric amounts of this cofactor bound to a unique lysyl residue; a protonated Schiff base is probably involved as indicated by the absorption maximum at 422 nm (Metzler, 1957; Heinert and Martell, 1963).

Other pyridoxal-5′-P-requiring bacterial decarboxylases include the inducible and constitutive arginine and ornithine decarboxylases (Blethen *et al.*, 1968; Wu and Morris, 1973; Applebaum, 1972) and the inducible enzymes for glutamic acid and tyrosine (Strausbauch and Fischer, 1970a; Cozzani *et al.*, 1970; Maruyuma and Coursin, 1968). At least two decarboxylases utilize pyruvate rather than pyridoxal-5′-P as an electron sink, namely, histidine decarboxylase from *Lactobacillus* 30a (Riley and Snell, 1968) and *E. coli* *S*-adenosylmethionine decarboxylase (Wickner *et al.*, 1970).

Pyridoxal-5′-P participates directly in catalysis and, therefore, its binding site can probably be considered to be part of the active site. However, since the first step in the catalytic reaction involves the displacement of the coenzyme from the protein by the substrate *via* a transaldimination reaction, the exact role of neighboring groups in the primary sequence surrounding the phosphopyridoxal lysyl residue is unclear. The

TABLE III: Alignment and Sequence of Peptides at the Pyridoxal 5′-Phosphate Binding Site of Lysine Decarboxylase.

Th-1	$\overrightarrow{\text{Val}}$-$\overrightarrow{\text{Ile}}$-$\overrightarrow{\text{Tyr}}$-$\overrightarrow{\text{Glu}}$-$\overrightarrow{\text{Thr}}$-$\overrightarrow{\text{Glu}}$-$\overrightarrow{\text{Ser}}$-$\overrightarrow{\text{Thr}}$-His-(ε-Pxy)Lys[a]
Na-3	(Val,Ile,Tyr,Glu)
Na-4	(Thr,Glu)
Na-1[b]	$\overrightarrow{\text{Ser}}$-$\overrightarrow{\text{Thr}}$-$\overrightarrow{\text{His}}$-(ε-Pxy)Lys
	His-(ε-Pxy)Lys
Ct-1	(ε-$\overrightarrow{\text{Pxy}}$)Lys-$\overrightarrow{\text{Leu}}$-$\overrightarrow{\text{Leu}}$-$\overrightarrow{\text{Ala}}$-$\overrightarrow{\text{Ala}}$-$\overleftarrow{\text{Phe}}$
Total sequence	Val-Ile-Tyr-Glu-Thr-Glu-Ser-Thr-His-(ε-Pxy)Lys-Leu-Leu-Ala-Ala-Phe

$\rightarrow$ subtractive Edman
$\leftarrow$ hydrazinolysis

[a] The presence of an (ε-Pxy)Lys residue at the COOH-terminal is consistent with the finding that no residue was released by extensive digestion with either carboxypeptidase A or B. [b] Na-1 was found to be a mixture of the tetra- and dipeptides as indicated.

use of group-specific and active-site reagents might help to answer this question.

Lysine and arginine decarboxylases are produced in *E. coli* under similar growth conditions, depending upon which particular substrate is present in the medium. They have identical subunit molecular weights of *ca.* 80,000 and both exist in dimeric and decameric forms. Their sedimentation coefficients, molecular weights, and appearance in the electron microscope are essentially indistinguishable (Blethen *et al.*, 1968; Boeker and Snell, 1968; Boeker *et al.*, 1969, 1971; Sabo *et al.*, 1974). They both require pyridoxal-5′-P and catalyze entirely analogous reactions presumably by the same mechanism.

The hypothesis that lysine and arginine decarboxylases are structurally related is further supported by the sequence data presented herein. Not only are the histidyl and seryl residues in the two pyridoxyl peptides coincident, but eight of the twelve residues are identical and two of the differences can be accounted for by single base changes in the corresponding genetic codons.

It is, of course, not surprising that little homology was found in sequences of the pyridoxal-5′-P binding sites previously determined since the enzymes were of varied origin and catalyzed totally different reactions. On the other hand, striking homologies are seen between β subunits of tryptophan synthetase from *E. coli* and *Pseudomonas putida*, and between arginine and lysine decarboxylases as described above (Table IV).

Another characteristic emerging from the sequence of pyridoxal-P binding sites of bacterial enzymes listed in Table IV is the change from predominantly charged and hydroxylated residues proximal to pyridoxyllysine to a largely nonpolar region on the distal side. This could, for example, be important in the formation of a hydrophobic pocket in which the cofactor might be bound.

Sequences obtained from the amino-terminal region of lysine and arginine decarboxylases suggest that the homology observed at the active site may extend to other regions. Though only two out of seven residues were identical, most changes resulted from conservative substitutions and both chains are rich in hydrophobic amino acids.

Further evidence for structural similarities between lysine and arginine decarboxylase stems from the observation that both native enzymes react identically with Nbs_2. The decamers fail to react at a significant rate while one sulfhydryl per monomer is readily titrated upon dissociation to dimers.

Two-dimensional peptide maps of lysine and arginine decarboxylase were carried out (see Methods) but no similarities were detected; this is not surprising in view of the size of the subunits and complexity of the peptide mixture. And, of course, small sequence differences could bring about large alterations in the chromatographic and electrophoretic behavior of the resulting peptides. Similarly, the extreme sensitivity of immunological reactions to minor differences in the structure of the antigens might explain the lack of cross-reactivity observed between the two decarboxylases.

Taken together, similarities in the chemical properties, subunit size and quaternary structure of lysine and arginine decarboxylases argue in favor of divergent evolution from a common ancestral protein. While these data are admittedly limited, it seems unlikely that their double pentameric structure would have emerged independently since nothing in the catalytic mechanism appears to dictate such an unusual architecture. These findings might be of particular interest because of the scarcity of information regarding this subject. While the internal homology observed between the left and

TABLE IV: Sequences at Pyridoxal 5′-Phosphate Binding Sites of Various Bacterial Enzymes.

Enzyme	Sequence	Source	Ref
Lysine decarboxylase	Val-Ile-Tyr-Glu-Thr-Glu-Ser-Thr-His-(ε-Pxy)-Lys-Leu-Leu-Ala-Ala-Phe	*E. coli*	
Arginine decarboxylase	Ala-Thr-His-Ser-Thr-His-(ε-Pxy)-Lys-Leu-Leu-Asn-Ala-Leu-Ser-Gln-Ala-Ser-Tyr	*E. coli*	*a*
Glutamic acid decarboxylase	Ser-Ile-Ser-Ala-Ser-Gly-His-(ε-Pxy)-Lys-Phe	*E. coli*	*b*
Tryptophan synthetase	Arg-Glu-Asp-Leu-Leu-His-Gly-Gly-Ala-His-(ε-Pxy)-Lys-Thr-Asn-Gln-Val-Leu-Gly-Gln-Ala-Leu-Leu	*E. coli*	*c*
Tryptophan synthetase	Arg-Glu-Glu-Leu-Asn-His-Thr-Gly-Ala-His-(ε-Pxy)-Lys-Val-Asn-Asn-Cys-Ile-Gly-Gln-Val-Leu-Leu	*P. putida*	*d*
Tryptophanase	Ser-Ala-Lys-(ε-Pxy)-Lys-Asp-Ala-Met-Val-Pro-Met	*E. coli*	*e*
Pyridoxamine–pyruvate transaminase	Val-Thr-Gly-Pro-Asp-(ε-Pxy)-Lys-Cys-Leu	*P. MA-1*	*f*

[a] Boeker *et al.* (1971). [b] Strausbauch and Fischer (1970b). [c] Fluri *et al.* (1971). [d] Maurer and Crawford (1971). [e] Kagamiyama *et al.* (1970). [f] H. Kolb, J. H. Hodsdon, R. D. Cole, and E. E. Snell, personal communication.

right halves of the bacterial ferredoxins (Tsunoda *et al.*, 1968), suggest that intracistronic duplication of genetic material has occurred in the course of evolution, little structural evidence to support the view that independent cistrons were generated by this process as a means of increasing enzymatic diversity.

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