Purification and Physical Properties of Inducible *Escherichia coli* Lysine Decarboxylase†

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ABSTRACT: Inducible lysine decarboxylase from *Escherichia coli* B is composed of subunits of mol wt 80,000, as determined by both hydrodynamic studies and electron microscopy. The native form of the enzyme appears to be a decamer (22.2 S, mol wt 780,000, at pH 7.0, μ 0.10) formed by the cyclic association of five dimers (7.8 S, mol wt 158,000 at pH 8.0, μ 0.02); its overall appearance is that of two stacked pentameric rings. Higher aggregates (*ca.* 60 S) result from the linear stacking of decamers to form rodlike particles of indefinite length. Conditions for optimum induction of the enzyme and its purification in large amounts are described. The method takes advantage of the heat stability and acid insolubility of the protein and yields 4 g of pure lysine decarboxylase/kg of wet bacteria. The final product is homogeneous by electrophoresis, isoelectric focusing, and ultracentrifugation. The enzyme decarboxylates hydroxylysine and *S*-aminoethylcysteine but not arginine, ornithine or a number of lysine analogs. The properties of *E. coli* lysine decarboxylase are compared to those of other amino acid decarboxylases, particularly arginine decarboxylase, which it resembles closely in terms of subunit size and quaternary structure.

Two groups of bacterial amino acid decarboxylases have been described: those that are produced constitutively in low amounts and carry out biosynthetic functions, and those which can be induced to high concentrations within the cell by their respective substrates. The former comprises L-ornithine, L-arginine, and *S*-adenosyl-L-methionine decarboxylases, which are involved in the biosynthesis of polyamines (Morris and Pardee, 1965, 1966; Wickner *et al.*, 1970) as well as diaminopimelic acid decarboxylase, which catalyzes the last step in the biosynthesis of lysine (White and Kelly, 1965). The inducible enzymes include the six decarboxylases for the L isomers of arginine, glutamic acid, histidine, lysine, ornithine, and tyrosine originally described by Gale (1946). Lysine decarboxylase was partially purified by Scher and Mallette (1954) who identified its heat-stable cofactor as pyridoxal-P.

While arginine and ornithine are degraded both by inducible and constitutive decarboxylases, the two types of enzymes appear to be structurally distinct and do not cross-react immunologically (Blethen *et al.*, 1968; Boeker and Snell, 1968; Boeker *et al.*, 1969, 1971; Wu and Morris, 1973; Applebaum, 1972). Lysine decarboxylase appears to occur only in bacteria and plants (Boeker and Snell, 1972).

Most of the decarboxylases utilize pyridoxal-P as an essential cofactor and all catalyze essentially the same reaction, differing only by their substrate specificity. *A priori*, one might expect that they all belong to a relatively homogeneous group of enzymes, arising perhaps from a common ancestral protein by divergent evolution. Of the six inducible enzymes, those for arginine and glutamic acid have been purified to homogeneity and extensively characterized. The former has a decameric structure with a subunit mol wt of 82,000 (Boeker and Snell, 1968; Boeker *et al.*, 1969) while glutamic acid decarboxylase is a hexamer with a subunit mol wt of 50,000 (Strausbauch and Fischer, 1970a). More recently the primary sequence of a segment of the pyridoxal-P binding site has been determined for both enzymes (Strausbauch and Fischer, 1970b; Boeker *et al.*, 1971). Little homology was evident except for a histidyl residue immediately adjacent to the phosphopyridoxyllysine group, and a serine three residues removed.

In order to further assess the relationship of structure to function among bacterial amino acid decarboxylases, a detailed study of lysine decarboxylase was undertaken. This enzyme was chosen because, like arginine decarboxylase, it utilizes a basic amino acid as substrate. Hence, a comparison between these two enzymes and glutamic acid decarboxylase might reveal certain common features among all the proteins, as well as differences perhaps dictated by the chemical nature of the substrates.

Materials and Methods

Uniformly labeled L-[^{14}C]lysine was obtained from New England Nuclear. Glyceraldehyde-3-P-dehydrogenase was provided by Dr. Philip Cohen of this laboratory. Phosphorylase was prepared as described previously (Sevilla and Fischer, 1969). Arginine decarboxylase was prepared according to the procedure of Boeker *et al.* (1969).

Activity Assay. Lysine decarboxylase was routinely assayed by a modification of the method of Morris and Pardee (1965) in which radioactive CO_2 released from [^{14}C]lysine (25 mM, *ca.* 4×10^6 dpm per mmol) is measured. The reaction was carried out at 37° in 0.3 ml of 0.2 M sodium acetate buffer (pH 5.7), containing 50 μM pyridoxal-P; it was initiated by adding 0.05 ml of an enzyme solution containing 0.15 mg of bovine serum albumin. Enzyme assays involving nonradioactive lysine analogs were carried out manometrically under

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

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similar conditions, *i.e.*, 2.5 ml of the reaction mixture was added to the main compartment of a Warburg flask and 0.5 ml of an enzyme solution containing 50 μg of bovine serum albumin to the side arm. Flasks were equilibrated at 37° for 15 min and the reaction was initiated by tipping.

For both assays, the reaction is proportional to the amount of enzyme added and linear until *ca.* 20% of the substrate has been decomposed. One unit of lysine decarboxylase represents the amount of enzyme catalyzing the release of 1 μmol of CO_2/min under the above conditions.

Protein Determinations and Absorbancy Index. Protein concentration was determined by the method of Lowry *et al.* (1951) on impure preparations (specific activity < 150 units/mg) and spectrophotometrically on more highly purified material, using an absorbancy index $A_{280}^{1\%}$ of 13.3. This value is the average of two independent measurements of protein concentration, obtained by either amino acid analysis or refractometrically in the ultracentrifuge, assuming a refractive index increment of 4.05 fringes/mg at 546 nm (Babul and Stellwagen, 1969). Values of 13.2 and 13.4, respectively, were obtained in 0.5 M sodium succinate (pH 6.0) and shown to be essentially the same in all buffers used.

Polyacrylamide gel electrophoresis of lysine decarboxylase (25–100 μg) was carried out in 5% gels prepared in Tris-HCl buffer (pH 7.0) according to Williams and Reisfield (1964); electrophoresis was run in Tris–diethylbarbituric acid buffer (pH 7.5) at 3 mA/gel. Protein was located by staining with 0.1% Amido Black. Decarboxylase activity was determined on *ca.* 1-mm gel slices dispersed with a glass rod in the assay buffer.

Polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate was carried out at 8 mA/tube according to Davis *et al.* (1967) in 5% gels on samples pretreated at 70° for 15 min in 1% dodecyl sulfate. Protein (5 μg) was applied for molecular weight determinations, and 20 μg for purity checks; gels were stained for 1 hr with Coomassie Brilliant Blue. Logarithmic plots of relative migration *vs.* molecular weight were obtained with the following markers: bovine serum albumin monomer and dimer (Tanford *et al.*, 1967), glyceraldehyde-3-phosphate dehydrogenase (Harrington and Karr, 1965; Harris and Perham, 1968), ovalbumin monomer and dimer (Castellino and Barker, 1968), and glycogen phosphorylase (Cohen *et al.*, 1971).

Isoelectric Focusing. After exhaustive dialysis against 1% glycine, 8.3 mg of protein was placed in the middle of a sucrose gradient containing 3% carrier ampholytes (pH 3–6) and 10 mM 2-mercaptoethanol in a 110-ml LKB electrofocusing column. Electrofocusing was carried out at 4° at 600 V for 10 hr, then at 900 V for an additional 42 hr. Approximately 100 fractions were collected and analyzed for enzyme activity, protein concentration, and pH. Electrofocusing was also carried out on 50 μg of enzyme in 10% polyacrylamide gels according to Wrigley (1968) using pH 3–10 carrier ampholytes.

Ultracentrifugation. Sedimentation experiments were performed at 10° in a Beckman Model E analytical ultracentrifuge equipped with an electronic speed control using double-sector cells with 12-mm optical paths. Four potassium phosphate buffers of differing ionic strength and pH were used, namely, buffer A, μ 0.02, pH 8.0; B, μ 0.04, pH 7.8; C, μ 0.10, pH 7.0; and D, μ 0.50, pH 6.0. All contained 1 mM EDTA.

Sedimentation equilibrium experiments were performed at 10° in a titanium AN-H rotor both by the conventional low-speed method using double-sector cells and by the high-speed procedure of Yphantis (1964) using six-channel cells. M_w was calculated from low-speed runs using interference photographs according to Van Holde and Baldwin (1958). Data reduction for high-speed runs was performed on a CDC 6400 computer using the program of Teller *et al.* (1969) which computes the point-average molecular weight moments as a function of protein concentration.

For ultracentrifugation in guanidine·HCl, samples were first dialyzed exhaustively against distilled water and lyophilized. Appropriate amounts were then dissolved in buffer A containing 6 M guanidine·HCl and 10 mM 2-mercaptoethanol. Yphantis cells were loaded as quickly as possible to minimize evaporation.

A partial specific volume of 0.739 ml/g, determined by amino acid analysis (see following publication), was used for molecular weight calculations (Cohn and Edsall, 1943) except for runs in guanidine·HCl where a value of 0.729 was employed (Hade and Tanford, 1967). The density of buffers containing guanidine·HCl was determined by pycnometry; that of the other buffers was obtained from International Critical Tables.

Electron Microscopy. Enzyme solutions (0.1–1.0 mg/ml) were sampled for negative staining by placing drops on freshly glow-discharged grids covered with carbon-coated Formvar films. Some grids were rinsed in the same buffer and transferred to buffered (0.05 M sodium phosphate) 1.5% glutaraldehyde for 10 min, adjusted to the same pH and ionic conditions as the sample. Samples were negatively stained with either 1% (w/v) aqueous sodium phosphotungstate (adjusted to pH 7.0) or 1% (w/v) aqueous uranyl acetate (not adjusted, pH 4.9). Stains were pulled off the edges of the grids with filter paper and the samples were dried with a stream of desiccated air. Specimens were examined in a Phillips EM 300 microscope at 60 kV with a 20-μ thin-metal condenser aperture. Magnification was determined with a diffraction grating replica displaying 2160 lines/mm (E. F. Fullam, Inc., Schenectady, N. Y.). Micrographs were recorded on Kodak Electron Micrograph film and measured directly with calipers or in a Nikon optical comparator.

Preparation of Apoenzyme. Pyridoxal-P was removed from the enzyme by dialyzing a solution of holoenzyme (10 mg/ml) for 4 days against four changes of potassium phosphate buffer, pH 6.0, μ 0.10, containing 1 mM EDTA, 0.1 M L-cysteine, and 0.05 M 2-mercaptoethanol, followed by dialysis against buffer without sulfhydryl derivatives.

Absorption spectra of lysine decarboxylase were determined on a Beckman DK-1 recording spectrophotometer. Enzyme was dialyzed against 0.1 ionic strength buffers containing 1 mM EDTA (potassium phosphate between pH 6 and 8 and Tris-HCl at pH 9).

Results

Conditions for both maximum induction and optimum yield of the enzyme during the purification were systematically investigated. The procedure yields a homogeneous material after *ca.* 30-fold purification of the crude bacterial extract.

Growth of E. coli B. Growth experiments and conditions for maximum induction of the enzyme are listed in Table I. In the final procedure, an inoculum of wild-type *E. coli* B was grown on a minimal medium (0.4% ammonium sulfate, 0.1% NaCl, 0.1% K_2HPO_4, 0.1% $MgSO_4$, 0.05% sodium citrate, and 0.1% glucose) in three successive steps at 37° as follows. Two 50-ml portions were first inoculated from the nutrient agar slants and allowed to grow for 24 hr with

TABLE I: Effect of Growth Conditions on Induction of Lysine Decarboxylase.

Variable[a]	Range	% Max. Sp Act.[a]
L-Lysine·HCl (%)	0.0	10
	0.05	48
	0.1	64
	0.2	83
	0.4	92
	0.8	100
Glucose (%)	0	44
	1	84
	2	100
Initial pH	7.0	22
	6.2	49
	5.5	82
	5.0	100[b]
Temperature (°C)	25	32
	37	100
Aeration	Vigorous	1
	None	100

[a] When one parameter was varied, the others were kept constant as follows: 0.4% $(NH_4)_2SO_4$, 0.1% NaCl, 0.1% K_2HPO_4, 0.1% $MgSO_4$, 0.05% sodium citrate, 0.8% L-lysine·HCl, 1% glucose, 1% nutrient broth, pH adjusted to 5.5 with H_3PO_4, 37°, no aeration, 9-hr growth. [b] Cell yield very low.

shaking. Cells were transferred to two 500-ml portions of culture medium and incubated for 15 hr, then both cultures were added to a 20-l. carboy containing 10 l. of medium and allowed to grow for an additional 9 hr with vigorous aeration through a sintered glass sparger.

The final inoculation suspension was added to a 100-l. New Brunswick fermentor, containing 90 l. of inducing medium (see Table I), and allowed to grow anaerobically for 9 hr at 37°. Approximately 140 g of wet cells was harvested in a large Sharples centrifuge.

Induction of enzyme approximately paralleled growth when bacteria were transferred from minimal to inducing medium. Since specific activity began to decrease early in stationary phase, growth time was carefully controlled. The specific activity also decreased if the cells were not used within a few days of harvest.

Preparation of Crude Extract. Freshly harvested cells were suspended in four volumes of 0.1 M sodium succinate (pH 6.2), containing 10 mM 2-mercaptoethanol, 1 mM EDTA, and 0.1 mM pyridoxal-P, and disrupted at 0° in 200-ml batches using a Branson sonifier, at a power setting of 5, for 20 min; unbroken cells and other particulate material were removed by centrifugation. Unless otherwise indicated, all operations were carried out at 4° and centrifugations at 16,000*g*.

Heat Treatment. Aliquots (200 ml) of crude extract were rapidly brought to 70° by swirling in an 80° water bath, and maintained at this temperature for another 5 min; the suspension was then cooled in an ice bath, centrifuged for 10 min, and the supernatant was collected.

Ammonium Sulfate Fractionation. The heat-treated solution was brought to 45% saturation with a saturated solution of ammonium sulfate containing 0.1 M sodium succinate (pH 6.2). After 30 min the precipitate was removed by a 20-min centrifugation and the supernatant was brought to 55% saturation in the same manner. After several hours, the yellow precipitate was collected by a second 20-min centrifugation and dissolved in 0.1 M sodium succinate (pH 6.2). Insoluble material was removed by centrifugation and discarded.

Acid Precipitation. Since lysine decarboxylase precipitates readily below pH 5.5, final purification was achieved by dialysis against 100 volumes of 50 mM sodium succinate (pH 5.2). Essentially all other proteins which copurified to this point, including arginine decarboxylase, remained in solution. The precipitated protein was collected by centrifugation for 15 min at 30,000*g* and washed twice with several volumes of dialysis buffer. The pure enzyme was redissolved in 0.1 M sodium succinate (pH 6.0), containing 1 mM EDTA, by heating briefly to 37°, and stored at −10° in this buffer, in the presence of 20% glycerol.

Crystallization. On rare occasions, lysine decarboxylase formed large square crystalline plates on standing at 4° for several months in Tris-HCl buffer, pH 9.0, μ 0.10. Since the process was not reproducible and did not increase the specific activity, it was not routinely attempted. A typical purification from 140 g of bacteria is summarized in Table II. The final specific activity was routinely between 900 and 1100 units per mg.

TABLE II: Summary of Lysine Decarboxylase Purification.

Step	Total Vol (ml)	Total Units ($\times 10^{-5}$)	Total Protein (g)	Sp Act. (Units/mg)	Recovery %
Crude extract	750	3.43	14.90	23	100
Heat-treated supernatant	650	2.96	2.80	106	86
Ammonium sulfate precipitate	44	5.18	1.60	329	151
pH 5.2 precipitate	22	4.50	0.41	1018	129

Purity of Lysine Decarboxylase Preparations. The final preparations were found to be homogeneous by a number of criteria. They showed less than 0.1% decarboxylase activity toward arginine, ornithine, and glutamic acid, indicating no contamination by these enzymes. Disc gel electrophoresis yielded a single protein band corresponding to the enzymatic activity (Figure 1) even when large amounts of protein were applied. Single bands were also obtained when the gels were run in the presence of dodecyl sulfate. The preparations appeared to be homogeneous in analytical isoelectric focusing experiments and gave a single symmetrical peak in the ultracentrifuge under conditions favoring a single state of aggregation (see below). Although a substantial amount of enzyme precipitated during electrofocusing on a sucrose gradient, coincident peaks of protein and enzymatic activity were observed at pH 4.6. Analytical isoelectric focusing in polyacrylamide gels gave a single protein band between pH 4 and 5.

Substrate Specificity. The enzyme exhibited Michaelis–Menten kinetics toward L-lysine. Among a number of substrate analogs tested (see Table III), *S*-aminoethyl-L-cysteine and δ-hydroxylysine (mixture of four isomers) were decar-

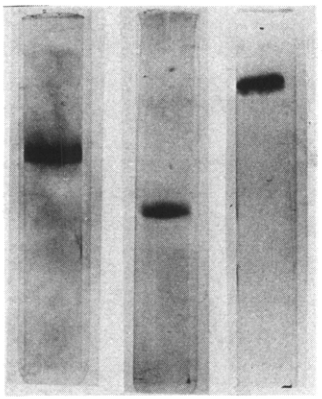

FIGURE 1: Polyacrylamide gel analysis of lysine decarboxylase: left, at pH 7.5; center, in the presence of 0.1% sodium dodecyl sulfate; right, analytical isoelectric focusing, from pH 3 to 10. Gels contained 25, 10, and 50 μg of lysine decarboxylase, respectively.

TABLE III: Substrates and Competitive Inhibitors of Lysine Decarboxylase.[a]

Substrates	K_m	Rel V_{max}
L-lysine	1.5	1.00
S-Aminoethyl-L-cysteine	3.4	0.15
δ-Hydroxylysine (DL and DL allo)	7.0	0.25
Competitive Inhibitors	**K_i**	
ϵ-Aminocaproic acid	1.8	
L-Lysine ethyl ester	8.6	
α-Aminopimelic acid	31.8	
1,5-Diaminopentane (cadaverine)	55.0	

[a] Neither substrate nor inhibitor: D-lysine, DL-α-amino-ϵ-hydroxycaproic acid, L-arginine, 1,4-diaminobutyric acid, L-alanine, glycine, L-norleucine, L-ornithine, glycine + *n*-propylamine.

boxylated at maximum rates of *ca.* 15 and 25% that observed for L-lysine, respectively.

pH dependence of enzymatic activity was determined at constant ionic strength; a bell-shaped curve, centered at pH 5.7, was obtained. Approximately 50% of the activity remained at pH 4.7 and 6.6. As determined by adding varying amounts of NaCl to the assay (carried out in 0.03 M sodium acetate or succinate buffer), the activity decreases with increasing ionic strength; a 45% inhibition was observed in 1 M NaCl.

Subunit Molecular Weight. Lysine decarboxylase subjected to polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate displayed a relative migration of 0.53 ± 0.01 in eight determinations, corresponding to a subunit molecular weight of 80,000 ± 3% when compared to a number of marker proteins (see Methods). Arginine decarboxylase, which was reported to have a subunit molecular weight of 82,000 (Boeker *et al.*, 1969), did not separate from lysine decarboxylase when a mixture of both was applied to the gel.

The subunit molecular weight was confirmed by high-speed sedimentation equilibrium in the presence of 6 M guanidine·HCl; values of 76,000, 82,000, and 84,000 were obtained for M_n, M_w, and M_z, respectively. These results are summarized in Table IV and a plot of molecular weight *vs.* concentration is illustrated in Figure 2.

TABLE IV: Summary of Molecular Weight Data Obtained in the Ultracentrifuge.

	M_w	M_z	Method	Rotor Speed
Monomer[a]				
6 M guanidine·HCl	82,000	84,000	H.S.[b]	22,000
Associated forms				
8S species	153,900	155,100	H.S.	14,000
	155,500	154,200	L.S.	6,800
22S species	759,700	797,800	H.S.	6,800
	730,800	770,700	L.S.	3,000

[a] A value of 80,000 ± 3% was obtained by polyacrylamide gel electrophoresis in the presence of dodecyl sulfate (see Results). [b] H.S. and L.S.: high- and low-speed sedimentation equilibrium, respectively.

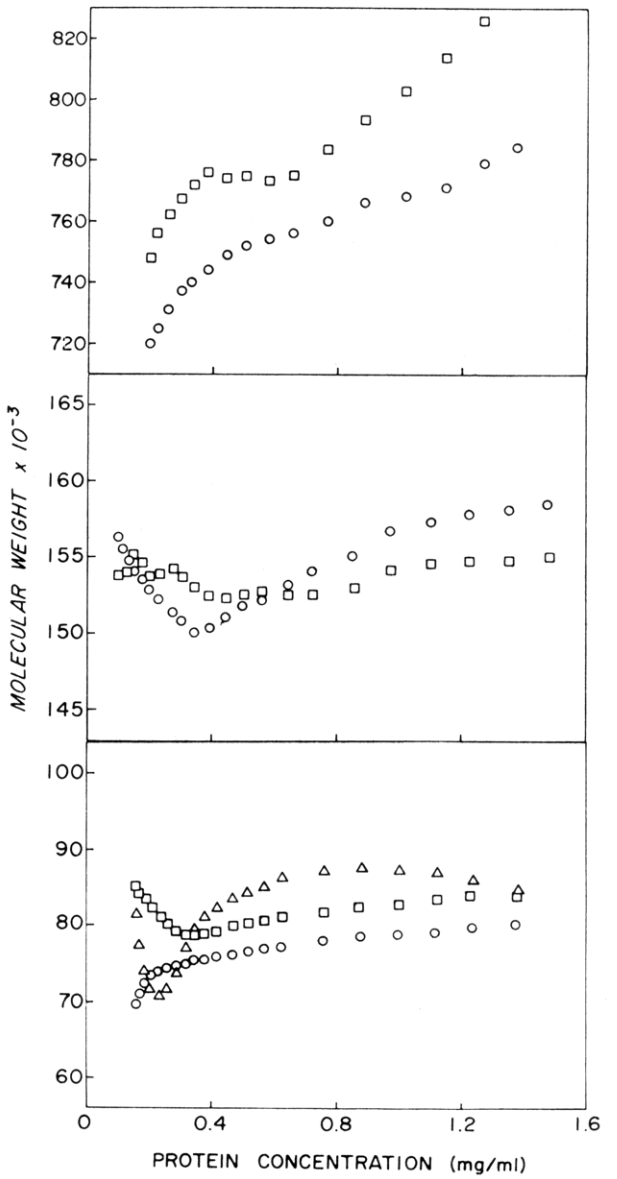

FIGURE 2: High-speed sedimentation equilibrium analysis: upper panel shows decamer, 22.2S species, in buffer C; center panel shows dimer, 7.8S species, in buffer A; lower panel gives results in 6 M guanidine·HCl. M_n (Δ), M_w (○), and M_z (□), number-, weight-, and *z*-average molecular weights, respectively.

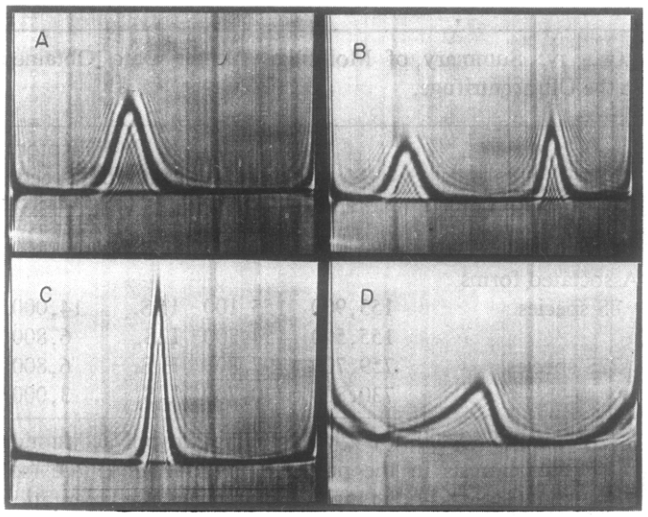

FIGURE 3: Sedimentation of lysine decarboxylase: (A) in buffer A, 7.8S species; (B) in buffer B, mixture of 7.8S and 22.2S species; (C) in buffer C, 22.2S species; (D) in buffer D, polymeric forms with peak corresponding to *ca.* 60 S. All samples contained 4 mg/ml of protein and were run at 10° with a rotor speed of 48,000 rpm.

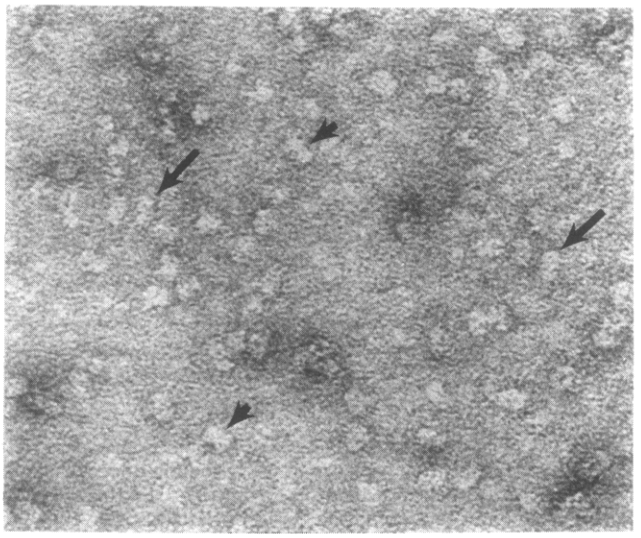

FIGURE 4: Lysine decarboxylase as the unfixed dimers, negatively stained with uranyl acetate. Many images are oblong structures (long arrows) but bilobed structures, suggestive of two identical subunits, are seen occasionally (arrowheads) (×300,000).

Sedimentation Coefficients. Lysine decarboxylase was found to associate or dissociate reversibly as a function of pH and ionic strength and therefore sedimented as a monodisperse preparation only under conditions favoring one particular state of aggregation. At pH 8.0, μ 0.02 (buffer A, see Methods) the enzyme had a sedimentation coefficient, $s^0_{20,w}$, of 7.8 S. As the pH was lowered and the ionic strength increased, the enzyme associated to a more rapidly sedimenting species until at pH 7.0, μ 0.10 (buffer C), the enzyme was entirely converted to a form with $s^0_{20,w}$ of 22.2 S. Under intermediate conditions, *e.g.*, pH 7.8, μ 0.04 (buffer B), a mixture of both 7.8S and 22.2S species was observed. If the pH were further decreased or the ionic strength increased, for example, to 6.0 and 0.50, respectively, the 22.2S species aggregated to a nonhomogeneous population of molecules with sedimentation coefficients as high as 70 S. Schlieren patterns for each of the above conditions are shown in Figure 3.

Molecular Weights of the Associated Forms of Lysine Decarboxylase. Both low- and high-speed sedimentation equilibrium experiments were performed on lysine decarboxylase following exhaustive dialysis against buffer A to give the 7.8S species; the values obtained for M_w and M_z by both methods (Table IV) are in excellent agreement. It was concluded from these data that the 7.8S species represents a dimer of the 80,000 molecular weight subunit. High-speed sedimentation equilibrium revealed a single molecular weight as a function of enzyme concentration, indicating that further association or dissociation was minimal under these experimental conditions (Figure 2). This result also indicated that charge effects were rather insignificant even though determinations were carried out at low ionic strength and at a pH of more than 3 units greater than the p*I* of the enzyme.

After exhaustive dialysis against buffer C to give the 22.2S species, low- and high-speed sedimentation equilibrium experiments were performed. The values given in Table IV for M_w and M_z are within 10% of each other. Since this species is a polydimer, it must contain an even number of subunits. The data are therefore most consistent with the view that the 22.2S species is made up of 10 subunits; that is, that it results from the association of five dimers. A plot of molecular weight *vs.* protein concentration (Figure 2) obtained from a high-speed sedimentation experiment shows some tendency for the decamer to dissociate or aggregate as enzyme concentration is lowered or increased, respectively.

Electron Microscopy. Structures with a regular size and shape were found in unfixed preparations of dimer in buffer A following staining with uranyl acetate (Figure 4). Dimensions were measured from *ca.* 50 photographs by determining the major axis and using the perpendicular direction as the minor axis. Values of 84 ± 18 and 62 ± 12 Å, respectively, were obtained, giving an axial ratio of 1.35; errors are expressed as ±2 std dev. A plot of major *vs.* minor axes for individual images gave a broad scatter about the mean values but no indication of subpopulations with respect to axial ratios. These results suggest that the dimensions of individual subunits are greater along the minor axis of the dimer than along its major axis.

Unfixed preparations of decamer in buffer C clearly showed *D*5 dihedral symmetry when stained either with uranyl acetate or sodium phosphotungstate (Figures 5); most images were pentagonal with five roughly circular equivalent substructures. In uranyl acetate each subunit had an apparent diameter of 64 ± 18 and of 69 ± 22 Å in phosphotungstate. The complete decamer had diameters of 168 ± 16 and 171 ± 18 Å, respectively, from the outer margin of one subunit to a line joining the outer margins of the opposite two. The central hole accessible to negative stain varied in diameter from 20 to 30 Å.

Edge views of the decamer served to establish the orientation of the dimer within the decameric unit as roughly perpendicular to the plane of the pentameric rings. The thickness of the decamer was 69 ± 10 and 65 ± 6 Å in the two stains, respectively. The value of 84 ± 18 Å, determined for the major axis of isolated dimers is consistent; not only are the standard deviations large enough to accommodate both values, but conformational changes may occur when the dimer is removed from the larger structure and spread on supporting films. Alternatively, the dimer may not lie exactly parallel to the fivefold axis; a few degrees of tilt would account for the differences in dimensions.

Individual images were examined for subunit detail by subjecting them to rotational reinforcement (Markham *et al.*,

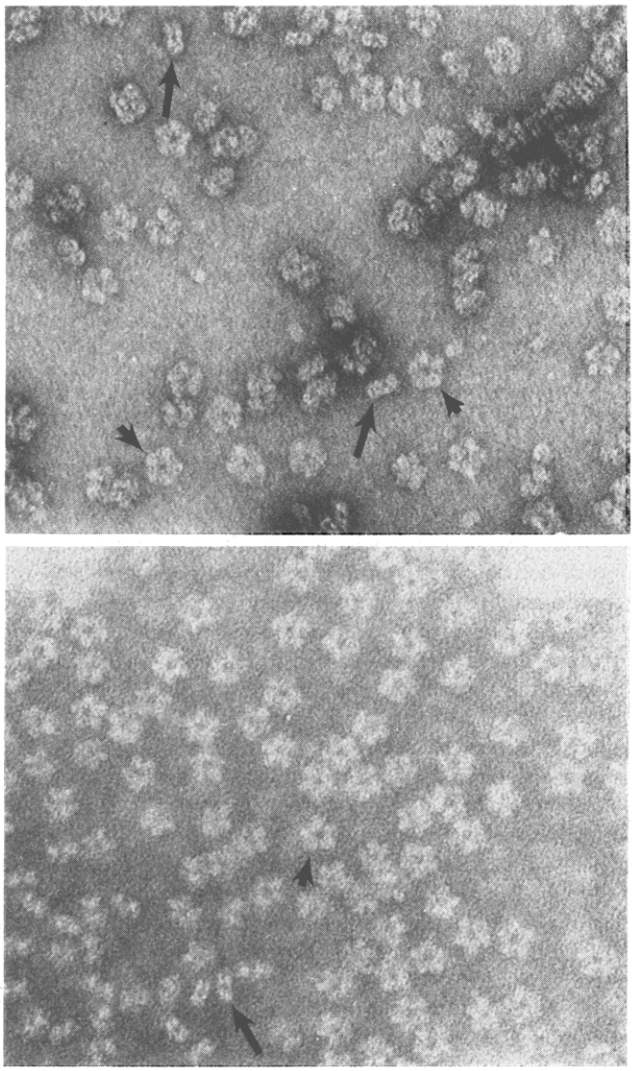

FIGURE 5: Decamers, unfixed and negatively stained with uranyl acetate (upper) and phosphotungstate (lower). Fivefold symmetry is seen in most images (arrowheads); edge views (long arrows) are occasionally found. An irregular linear aggregate (upper right) formed during staining (×231,000).

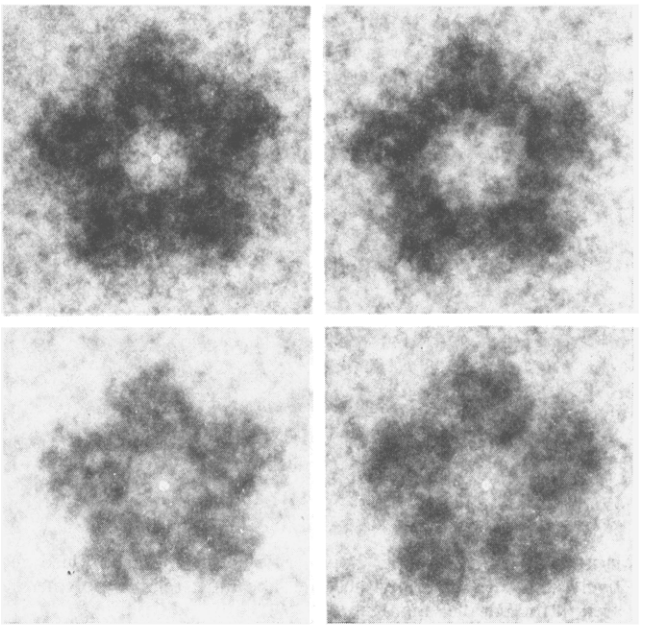

FIGURE 6: Examination of unfixed decamers stained with phosphotungstate for subunit detail by rotational reinforcement of fivefold symmetry; these appear as positives because they are printed as negatives of negatively stained images. Fourfold, sixfold, and tenfold reinforcement showed no rotational periodicity (×2,000,000).

1963). Only fivefold rotation showed enhancement of subunits (Figure 6). Structural detail was occasionally seen, but no consistent pattern emerged from the analysis of 40 molecules in this manner.

Higher aggregates, brought about by increasing the salt concentration or decreasing pH, consisted of periodic linear polymers of decamers with some additional tendency toward parallel aggregation (Figure 7). Each polymer contained an even number of pentameric layers in which alternate spacings, *i.e.*, those between decamers, were more distinct than those between layers belonging to the same molecule. The thickness of the decamer could be accurately measured in these aggregates; a period of 74 ± 2 Å was obtained from five segments, each five periods in length. The straightness of their margins and apparent precision of each aggregate in a bundle suggested that these are highly ordered crystallites with a fivefold axis of symmetry, coincident with the fivefold axis of the component decamers. There must also exist, therefore, five local twofold axes in the plane between adjacent decamers.

Activity of Various Molecular Forms. The most active form of the enzyme was shown to be the decamer. This was determined by filtration of the decarboxylase on a column of Sepharose 4B under conditions where the enzyme is maximally active, *i.e.*, those of the routine assay (pH 5.7, 37°, 1 μg/ml of enzyme). In this experiment, the substrate was replaced by a competitive inhibitor, ε-aminocaproic acid, to prevent reaction. The results shown in Figure 8 were obtained regardless of whether dimeric, decameric, or aggregated lysine decarboxylase was used as starting material, or whether the substrate analog was included or omitted from the buffer. These results indicate that the dimeric and aggregated forms are either inactive or not present under these conditions. Since the dimer, when assayed at 1 mg/ml in buffer A (μ 0.02, pH 8.0) displays at least 1% of maximum activity, it is most probably absent in this experiment where optimal assay conditions are used.

Absorption Spectrum. Lysine decarboxylase displayed absorption peaks both in the ultraviolet and visible regions with maxima at 278 and 422 nm. The 278/422 nm absorbance ratio is about 20 to 1. Both were pH independent between pH 6.0 and 9.0; spectra below pH 6 could not be determined due to precipitation of the sample. The 422-nm absorbance was absent from the apoenzyme, but could be restored by addition of stoichiometric amounts of pyridoxal-P.

Properties of Lysine Apodecarboxylase. When apoenzyme was subjected to analytical ultracentrifugation under conditions described for the holoenzyme, the same sedimentation coefficients were observed, within experimental error (*ca.* 5%). Dimers, decamers, and mixtures of these two forms as well as high molecular weight aggregates could be observed under the appropriate conditions.

Lysine apodecarboxylase differed from the holoenzyme with regard to heat stability—the apoenzyme was inactivated within minutes above 60° while the holoenzyme was completely stable for 20 min at 70° and still retained 60% of its activity at 80° for this period of time. Together, these results indicate that coenzyme binding stabilizes the native conforma-

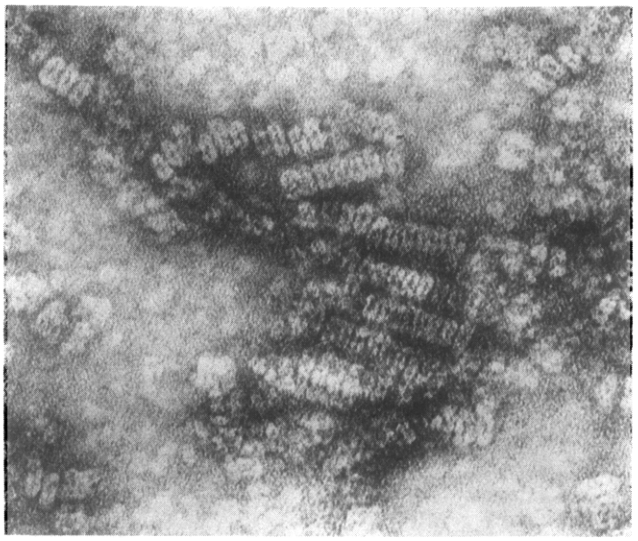

FIGURE 7: Unfixed higher polymers of lysine decarboxylase negatively stained with uranyl acetate. The most regular polymers are seen in the parallel aggregates.

tion of the enzyme but does not cause large alterations in the overall size, shape, or surface properties of the molecule.

Discussion

The purification described here for lysine decarboxylase is rapid and simple since it relies mainly on the heat stability of the enzyme and its insolubility at moderately acidic pH; it is carried out without the use of columns and hence can easily be scaled up. An unusual feature of the procedure is an increase in total enzymatic activity at the ammonium sulfate precipitation step, resulting in greater than 100% recovery of units. This increase does not appear to be due to changes in the state of aggregation of the enzyme since it was not affected by preequilibration in buffer C (μ 0.10, pH 7.0), conditions known to favor decamer formation. Possible causes (for instance, removal of an inhibitory substance or an irreversible change in conformation induced by high salt conditions) were not further investigated.

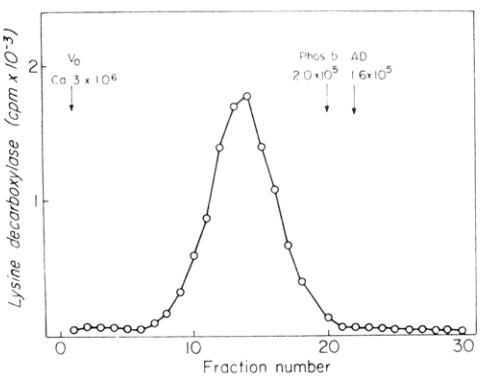

FIGURE 8: Gel filtration of lysine decarboxylase on Sepharose 4B under assay conditions. The initial protein concentration was 10 ng/ml; the final, approximately 1 ng/ml. The enzyme was eluted at 37° with 0.2 M sodium acetate (pH 5.7), containing 50 μM pyridoxal-P and 25 mM ϵ-aminocaproic acid; fractions were 1 ml. Blue dextran (mol wt $> 3 \times 10^6$), phosphorylase *b* (phos *b*, mol wt 200,000) and the arginine decarboxylase dimer (AD, mol wt 160,000) were used as standards. Neither omission of the substrate analog nor the aggregation state of the starting material (dimer, decamer, or aggregate) altered the elution profile.

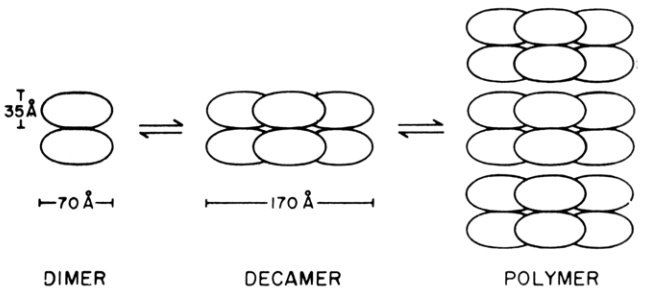

FIGURE 9: Model for the self-association of lysine decarboxylase. The decamer, probably the native form, is composed of five dimers linked by heterologous interactions. The two pentameric rings forming the decamer are held together by bonds which are probably isologous. The interactions between decamers in the aggregated state are probably also isologous, but are weaker than those between pentamers.

While its function is still unclear, there is no indication that lysine decarboxylase participates in a catabolic pathway yielding utilizable energy, since the product of its reaction, cadaverine, is not further metabolized but is excreted by the cell (Hanke and Koessler, 1924). Furthermore, induction is not subject to catabolite repression, and maximum enzyme levels are produced when cells are grown at high concentrations of glucose. Since the net result of amino acid decarboxylation and excretion of the corresponding amine is the expulsion of a proton from the cell, Gale (1946) proposed that bacteria might use this reaction as a means of increasing intracellular pH when the organism is grown in acidic media. Later, Guirard and Snell (1964) suggested that this mechanism might also serve to control the intracellular concentration of CO_2, required in a variety of metabolic reactions, which would otherwise be exceedingly low under acidic conditions. Consistent with both hypotheses is the observation by Becker (1967) that an *E. coli* mutant lacking the inducible arginine decarboxylase grows very poorly in an enriched medium at pH 5.1 but normally at pH 6.1. Exogenous polyamines did not stimulate growth, suggesting that it was not the decarboxylated product of the reaction which was necessary for growth at low pH. While these proposals are reasonable, compelling evidence for them is not available and the possibility of other functions remains open.

Electron micrographs together with molecular weight and sedimentation data provide the basis of the model shown in Figure 9 for the self-association of lysine decarboxylase. They indicate that the enzyme is a polydimer exhibiting fivefold symmetry; the resulting decamer reversibly dissociates into five dimers or further aggregates to higher molecular weight species which appear as stacks of decamers in the electron microscope.

Since the spacing between the two pentameric rings which form the decamer seems to be closer than that between two decamers, different kinds of interactions are probably involved. This is further supported by the observation that disruption of the polymeric species during preparation of samples for the electron microscope occurs preferentially between every other pentameric layer (see Figure 7) and by the differential susceptibility of these bonds to pH and ionic conditions. As suggested for arginine decarboxylase (Boeker *et al.*, 1969), bonding within the dimer is probably isologous or "head-to-head" (Monod *et al.*, 1965). If so, the weaker association between decamers must also be isologous, but of a second type. Boeker *et al.* (1969) have noted that ring structures containing an odd number of identical sub-

units cannot result from isologous interactions. Therefore interactions between dimers and within the ring must be heterologous or "head-to-tail." Again as postulated for arginine decarboxylase, interactions within dimers are probably largely hydrophobic since they are not affected by variations in pH or ionic strength. By contrast, the intra-ring bonds are sensitive to these conditions and are probably at least partially ionic. It should be noted, however, that association of lysine decarboxylase increases with ionic strength, consistent with the interpretation that a charge repulsion must be overcome for attraction to take place. The reverse relationship is more common for subunit interactions (Frieden, 1971). Since variations in pH and ionic strength can also affect interaction between decamers in the aggregated state, they too are likely to be ionic in nature.

Some chemical and steric features of the active site of lysine decarboxylase can be deduced by the use of compounds which act either as substrates or competitive inhibitors (Table III). *A priori*, one might have expected that the covalent aldimine bond formed between pyridoxal-P and the α-amino group of the substrate would contribute much of the energy needed to stabilize the enzyme–substrate complex. Surprisingly, this appears not to be the case since ϵ-aminocaproic acid, which lacks an α-amino group, is a very good competitive inhibitor, binding approximately as well as the substrate. On the contrary, binding appears to depend mainly on the structure of the side chain, since *all* compounds which interact specifically with the enzyme possess an *n*-butylamino α substituent, except *S*-aminoethyl-L-cysteine in which a sulfur atom replaces one of the methylene bridges. L-Norleucine, in which the ϵ-amino group is replaced by a hydrogen atom, ornithine (which can be viewed as lysine with a side chain shortened by one methylene group), as well as L-alanine and glycine, are neither decarboxylated nor bound to the active site.

A definite structural relationship seems to exist between inducible lysine and arginine decarboxylases (Table V). Both catalyze essentially the same reaction and require pyridoxal-P as cofactor. Most striking, however, are similarities in their exceptionally large subunit molecular weight and unusual quaternary structures. The subunits are not separable by electrophoresis in sodium dodecyl sulfate and form dimeric and decameric structures that are indistinguishable in the ultracentrifuge and electron microscope; the latter structure is so far unique to these enzymes. Conditions for association and dissociation are very nearly identical and somewhat atypical, both enzymes tending to dissociate as the net charge of the molecules is decreased. Both have low isoelectric points and pH optima, and K_m values and specific activities of the same order of magnitude. Among the differences are the fact that substrate analogs cause aggregation of arginine decarboxylase from dimer to decamer, and intermediate forms can be seen in the ultracentrifuge (Boeker *et al.*, 1969). This behavior has not been observed for lysine decarboxylase, perhaps because dissociation of this enzyme requires high pH where binding of analogs might not occur due to neutralization of the ϵ-amino group. Lysine—but not arginine—decarboxylase, readily aggregates to higher molecular weight linear polymers. Lysine decarboxylase from a distantly related bacterium, *Bacillus cadaveris*, appears to have properties similar to the *E. coli* lysine and arginine decarboxylases (Soda and Moriguchi, 1969) as shown in Table V.

TABLE V: Comparison of Lysine and Arginine Decarboxylases.

Property	Decarboxylase		
	Lysine	Arginine[a]	Lysine[b]
Source	*E. coli*	*E. coli*	*B. cadaveris*
Enzyme in crude extract (%)	6	6	2
Specific activity	1000	400	90
K_m (mM)	1.5	0.6	0.4
pH optimum	5.7	5.2	5.8
Subunit molecular weight	80,000	82,000	
Molecular weight (native)	780,000	820,000	1,000,000
Subunit structure	Dimer, decamer, aggregates	Dimer, decamer	
$s_{20,w}$	7.8, 22.2	8.0, 23.3	21.1

[a] Boeker *et al.* (1969). [b] Soda and Moriguchi (1969).

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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.