

Colorimetric Assay for Lysine Decarboxylase in *Escherichia coli*

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A new assay is described for lysine decarboxylase. It is rapid and reproducible in assaying large numbers of samples, a situation in which earlier methods were less convenient. The new method is valuable in the study of peptide fractions and amino acid mixtures which stimulate induction of lysine decarboxylase. It may be useful for work on enzyme structure and modification, genetics, and kinetics.

Methods available for the determination of lysine include paper chromatography, electrophoresis, ion-exchange chromatography, and microbiological assays (1, 8), as well as a manometric technique (5) which measures the CO₂ evolved during decarboxylation of the amino acid. For study of the stimulation of the biosynthesis of lysine decarboxylase by peptides (4), these methods either were not suitable for assaying a large number of samples or were not sufficiently reproducible. A colorimetric method (2) for the measurement of cadaverine formed by decarboxylation of lysine was found to be precise but time-consuming. Therefore, a simpler method was sought.

Based on data by Gilvarg (3) and Shimura and Vogel (6), we developed a rapid, simple, and reproducible colorimetric assay for lysine decarboxylase which is described in this report.

MATERIALS AND METHODS

Microorganism. *Escherichia coli* B was used throughout this investigation. Stock cultures were carried on nutrient agar slants, transferred weekly, and subcultured for experimental use by inoculation of 10 ml of glucose-salts medium (7) with a loop of cells from a slant. This suspension was incubated at 37 C with shaking until the turbidity indicated 5×10^8 cells per ml, which usually took 4 to 5 hr.

Enzyme induction. The medium for induction of enzyme biosynthesis was prepared from three stock solutions. Solution A contained 4.0 g of Na₂HPO₄·12H₂O and 5.0 g of KH₂PO₄ in 100 ml of distilled water. Solution B contained 10.0 g of NH₄Cl, 5.0 g of NaCl, and 4.1 g of MgSO₄·7H₂O in 100 ml of distilled water. Solution C contained 8 g of glucose, 4 g of L-lysine hydrochloride, and 8 g of an acid hydrolysate of casein (Hy-Case, Sheffield Chemical) in 315 ml of distilled water. Stock solutions A and B were separately sterilized at 120 C. Stock solution C was steri-

lized by filtration through a membrane filter (type GS, 0.22 μm pore size; Millipore Corp., Bedford, Mass.) A 20-ml amount of solution A, 1.1 ml of solution B, and 78.9 ml of solution C were combined aseptically. The acid hydrolysate of casein was omitted as a negative control for enzyme induction. A 1.0-ml sample of the stock cell suspension in glucose-salts medium (5×10^8 cells per ml) was inoculated into 100 ml of induction medium and shaken for 12 hr at 37 C. After the initial increase in lysine decarboxylase activity up to about 8 hr, the level of enzyme did not change for at least 5 more hours.

Preparation of enzyme extracts. This induction system containing 6.3×10^8 cells per ml was centrifuged at 5 C and $3,000 \times g$ for 10 min. The cells (6.3×10^{10} , dry weight, 19.4 mg) were washed by suspension in 100 ml of a cold 0.9% NaCl solution and recentrifuged. Washed cells were suspended in 85 ml of 0.02 M phosphate buffer (pH 7.0). A 10-ml amount of this cell suspension containing 2.3 mg of cells in a glass tube (25 by 75 mm) was placed in an ice bath and treated for 30 sec with a flat-tipped, 0.5-inch (1.27 cm) step-horn of a Branson Sonifier at an output of 2.5 amp. The cells were then shaken for 1 min in an ice bath, and this treatment was repeated four times. The resulting suspensions were centrifuged at 4 C for 20 min at $4,000 \times g$ to remove debris and unbroken cells. Supernatant liquid containing 0.069 mg of protein per ml was taken as the enzyme extract. Protein was determined by the method of Lowry et al. (3a) by using bovine serum albumin as the reference standard.

RESULTS AND DISCUSSION

Absorbance produced by reaction of lysine with ninhydrin (Fig. 1) may be used as a standard curve for the assay method. Cadaverine is without significant effect under the assay conditions.

The influence of substrate concentration upon enzyme activity is shown in Fig. 2. Lysine decarboxylase was most active at a final lysine concentration near 4×10^{-3} M. With the larger amounts of enzyme, lysine concentration became

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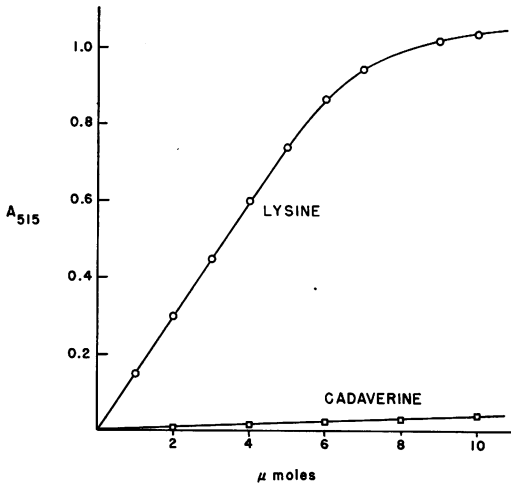


FIG. 1. Response of lysine and cadaverine to ninhydrin in phosphoric acid. Lysine or cadaverine in 0.5 ml of water was treated with 0.5 ml of 3 M HCl and 0.5 ml of 5% ninhydrin in methyl cellosolve (w/v) and heated at 100 C for 1 hr. The color reaction was stabilized by diluting with 4 ml of 15 M H_3PO_4 .

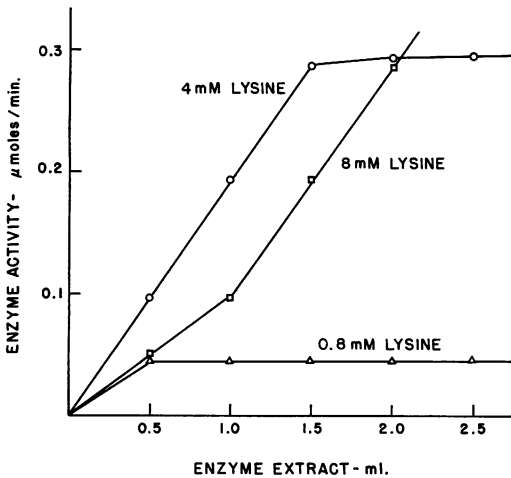


FIG. 2. Optimal concentration of lysine in the assay. Enzyme extract and buffer (0.02 M phosphate, pH 6.0) totaled 2.5 ml. Pyridoxal phosphate (0.2 ml of 4×10^{-4} M) and lysine (0.5 ml of 0.05 M, 0.025 M, or 5×10^{-3} M) were added and incubated 30 min at 37 C. Color was developed as for Fig. 1.

limiting, and it was necessary to rerun with more dilute enzyme. Figure 2 suggests that a high substrate-enzyme ratio inhibited enzymatic activity, a behavior which may indicate allosteric characteristics for the enzyme.

Although added pyridoxal-5-phosphate increased enzyme activity only slightly, the co-

enzyme was added routinely to the assay system to make certain that it did not become limiting with extracts containing higher enzyme levels or when the fractionation of extracts was monitored.

Figure 3 shows that the optimal pH for the assay was near pH 7.0 and resembled other decarboxylases in this respect (6). This pH optimum

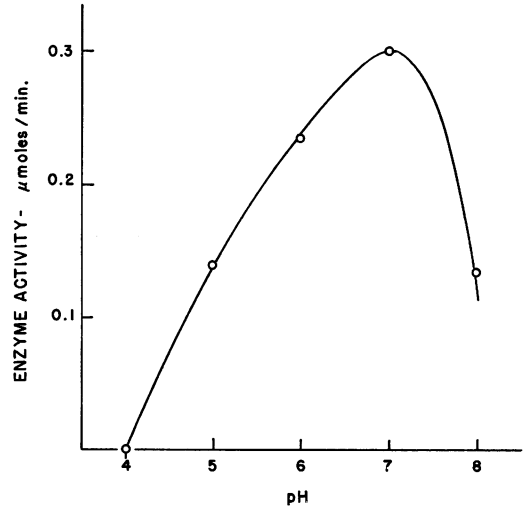


FIG. 3. Optimal pH for the lysine decarboxylase assay. One milliliter of unbuffered enzyme extract, 1.5 ml of 0.02 M phosphate buffer (pH 5, 6, 7, or 8) or of 0.02 M citrate buffer (pH 4), 0.2 ml of 4×10^{-4} M pyridoxal phosphate, and 0.5 ml of 0.025 M lysine were incubated for 30 min at 37 C. Color was developed as for Fig. 1.

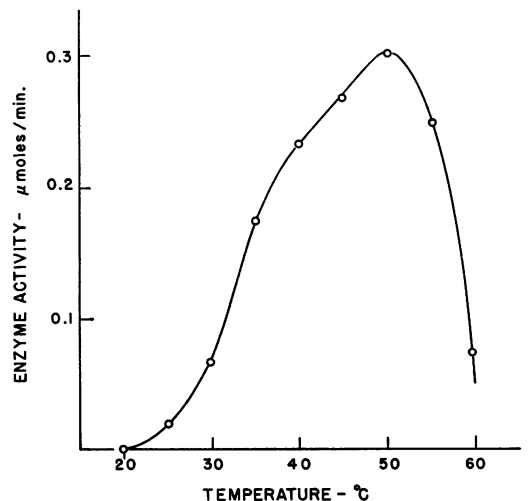


FIG. 4. Optimal temperature for lysine decarboxylase assay. See Fig. 3 for conditions, except that the assay system and enzyme extract were buffered at pH 7.0.

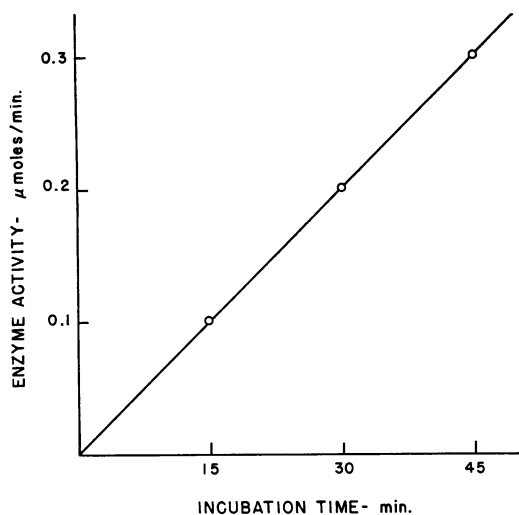


FIG. 5. Time course of the decarboxylation assay. See Fig. 3 for the conditions, except that the assay system was buffered at pH 7.0 and incubated at 37 C.

is slightly higher than the pH 6.0 reported (5) for the manometric assay.

Although the optimal temperature of the enzyme assay was 50 C (Fig. 4), for laboratory convenience an assay temperature of 37 C was employed. Figure 5 shows that enzyme activity at 37 C was a linear function of the time of incubation up to at least 45 min. Incubation for 30 min was chosen as a convenient interval.

For routine use the colorimetric assay involved incubation of enzyme in 0.0039 M lysine at 37 C and pH 7 for 30 min. Its performance is compared in Table 1 with that of the manometric method. The relevant standard deviation of the new colorimetric assay method showed it to be considerably more reproducible than the manometric method.

On the basis of these data the following procedure is recommended.

Lysine decarboxylase assay. The assay mixture contained 0.5 to 2.5 ml (0.035 to 0.173 mg) of enzyme extract in 0.02 M phosphate buffer (pH 7.0) with additional 0.02 M phosphate buffer (pH 7.0) to bring the volume to 2.5 ml, 0.2 ml of 4×10^{-4} M pyridoxal phosphate, and 0.5 ml of 0.025 M L-lysine hydrochloride solution. The reaction mixture was incubated at 37 C for 30 min. Enzymatic reaction was stopped by addition of 0.5 ml of 3 M HCl, and the color was developed by addition of 0.5 ml of 5% (w/v) ninhydrin in methyl cellosolve, followed by heating the foil-covered tubes in a boiling water bath for 1 hr. To this solution was added 4 ml of 15 M phosphoric acid. Ab-

TABLE 1. Reproducibility in the colorimetric and manometric assays for lysine decarboxylase^a

Method	Enzyme activity	SD ^b	Relative SD
	μmole/min		%
<i>Colorimetric</i>			
Enzyme extract (ml)			
0.5	0.132	5.3×10^{-3}	4
1.0	0.268	3.6×10^{-3}	1.4
1.5	0.355	2.48×10^{-3}	0.7
2.0	0.367 ^c	2×10^{-3}	0.5
2.5	0.369 ^c	1.3×10^{-3}	0.3
<i>Manometric</i>			
Incubation time (min)			
8	0.304	0.019	6.2
14	0.298	0.026	8.7
20	0.280	0.014	5.0

^a Each activity value was the average for five individual samples of extract. Assay conditions were those of Fig. 5, except that incubation was 30 min for the colorimetric assay, and the concentration of lysine was 5×10^{-3} M for manometric assay at pH 6.0 with 1 ml of enzyme extract. Cells from 50 ml of incubation medium were washed, suspended in 40 ml of buffer, and disrupted.

^b Standard deviation.

^c Values represent complete decarboxylation of the lysine present in the assay.

sorbance was read with a Beckman model DU spectrophotometer at 515 nm, and was converted to micromoles of residual lysine by use of a standard curve. Lysine was averaged for two blanks run without enzyme included. Enzyme activity was reported as micromoles of lysine decarboxylated per minute obtained by subtracting residual lysine from the blank value.

ACKNOWLEDGMENT

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