Studies on Amino Acid Decarboxylases in *Escherichia coli*

By ALEXANDER LAWSON AND A. G. QUINN
Royal Free Hospital School of Medicine, London, W.C. 1

(Received 2 March 1967)

The isolation of highly active preparations of glutamate decarboxylase, arginine decarboxylase and histidine decarboxylase from *Escherichia coli* is described. These preparations showed strict specificity, and were in each case resolved into apo- and co-enzyme as shown by the significant restoration of activity that took place on addition of pyridoxal 5-phosphate.

Smith & Waygood (1961) reported the resolution of glutamate decarboxylase from a plant source into apo- and co-enzyme. This finding lent support to the view held by Umbreit & Gunsalus (1945) that all amino acid decarboxylases are PLP*-dependent, but the possibility that this might not be so, first discussed by Gale (1946), still exists for glutamate decarboxylase and histidine decarboxylase from bacterial sources, particularly in view of the publications by Rosenthaler, Guirard, Chang & Snell (1965) and Cozzani (1965). The former workers maintain that a histidine decarboxylase extracted from *Lactobacillus* is a simple protein as shown by its u.v. spectrum, with no trace of PLP, and further that PLP does not increase the activity. This finding is supported by Mardashev & Semina (1964), who failed to find evidence for a requirement of PLP in histidine decarboxylase from *Micrococcus*. Cozzani (1965) claims that a glutamate decarboxylase extracted from *Clostridium welchii* is likewise not activated by PLP, though showing a u.v. spectrum characteristic of PLP-containing enzymes. These recent findings are not in agreement with the earlier work of Umbreit & Gunsalus (1945), who provided evidence that glutamate decarboxylase from *Escherichia coli* required PLP as coenzyme. Other workers have also shown this to be so for the enzyme from mammalian tissues (Roberts & Frankel, 1951).

Najjar & Fisher (1954) and Shukuya & Schwert (1960) purified glutamate decarboxylase from bacterial sources, but made no attempt to resolve the enzyme. However, the latter authors did record an enhancing effect of PLP on their preparation. Rodwell (1953) interpreted his failure to resolve histidine decarboxylase as being due to an unusually strong affinity between apo- and co-enzyme rather than to a non-requirement for the coenzyme.

Guirard & Snell (1954), having shown that long incubation of resting cells of *E. coli* with pyridoxine

enhanced their histidine decarboxylase activity, found that the activity of cell-free extracts of the enzyme after dialysis could be partly restored by PLP. Simultaneous addition of Fe^{3+} ions gave almost complete restoration. Eggleston (1958) also showed the enhancing effect of PLP, but not that of the metal ion. The histidine decarboxylase of mouse mastocytoma prepared by Ono & Hagan (1959) was also PLP-dependent.

Gale (1946) reported that arginine decarboxylase was difficult to resolve. Since then little work has been done on this bacterial enzyme. However, the work of Bonavita & Scardi (1958) suggested that the PLP might play a somewhat different role from that normally accepted for decarboxylation, a possibility that was also raised by Gonnard (1962) and Makino, Ooi, Matsuda & Kuroda (1962), who worked with brain glutamate decarboxylase and found that derivatives of PLP formed by condensation with carbonyl compounds activated the enzyme at low concentrations.

Although there seems little doubt that these enzymes are strictly substrate-specific, preliminary work on induction of various decarboxylases in *E. coli* did not at first confirm strict specificity. *E. coli* grown in a minimal medium with one amino acid decarboxylated other amino acids even after subculture. There are reports by Ekladius, King & Sutton (1957) and Haughton & King (1961) of non-specificity of a leucine decarboxylase from *Proteus vulgaris*; this enzyme was also capable of decarboxylating valine, norvaline, isoleucine and α -amino- γ -butyric acid. There are also a number of isolated references by other workers to non-specificity in decarboxylases; in particular Umbreit & Heneage (1953) reported on a glutamate decarboxylase capable of breaking down β -hydroxyglutamic acid. Roberts & Frankel (1951) have noted the breakdown of glutamine by a glutamate decarboxylase preparation. Linstedt (1951) has also shown lysine decarboxylase to decarboxylate

* Abbreviation: PLP, pyridoxal phosphate.

hydroxylysine. The work of Lovenberg, Weissbach & Udenfriend (1962) on certain kidney amino acid decarboxylases raised some doubts of their specificity, particularly for the aromatic amino acids and histidine. Ganrot, Rosengren & Rosengren (1961) and Schayer & Sestokas (1965) have shown a histidine decarboxylase from mammalian tissue to be non-specific. In view of this conflicting evidence it was decided to investigate highly purified preparations of arginine decarboxylase, glutamate decarboxylase and histidine decarboxylase to re-examine the question of cofactor dependence and, for glutamate decarboxylase, the specificity; it was decided also to contrast some of their properties such as pH optima and u.v. spectra.

METHODS

Growth of organisms. Cultures of *E. coli*, strains N.C.T.C. 86 (Gale, 1940) and N.C.I.B. 8545 (Kornberg, Phizackerly & Sadler, 1960), were grown on nutrient agar. Stock cultures were transferred monthly, grown at 30° and stored at 4°. For the preparation of enzymes, 2–4 l. batches of culture were grown, a 5% inoculum being used. In the preparation of arginine decarboxylase the minimal medium of Davis & Mingioli (1950) was used with added arginine (5%, w/v). In the preparation of histidine decarboxylase, the medium of Aiso, Yanagisawa, Toyoura, Fujita & Toyoura (1955) was used.

Chemicals. Throughout the experimental work, chemicals used were of A.R. grade. The ammonium sulphate for protein fractionation was that which is specially prepared by British Drug Houses Ltd., Poole, Dorset, for enzyme studies. The protamine sulphate and amino acids were of 'Biochemical' grade from British Drug Houses Ltd.; DEAE-cellulose, alumina C_γ gel (aged) and pyridoxal 5-phosphate were obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks.

Estimation of enzymic activity. The decarboxylase activity at the optimum pH of the enzymes was determined at 37° by the Warburg technique (Umbreit, Burris & Stauffer, 1961), and the measurements were made in an atmosphere of air. For glutamate decarboxylase, the pH used was 4.0 for cells and 3.7 for cell-free extracts; for arginine decarboxylase, pH 4.5 for cells, pH 4.9 for cell-free extracts; for histidine decarboxylase, pH 4.0 for both cells and cell-free extracts. Each flask contained a 2.5 ml. sample of the buffered enzyme source in the main compartment and 0.5 ml. of the substrate (0.05 M) in the side arm. The initial rate of evolution of carbon dioxide was measured without correction for its solubility in the buffer. Activities were usually calculated as Q_{CO_2} (μl. of CO₂/mg. dry wt. of protein/hr.).

Protein estimation. This was carried out by the method of Lowry, Rosebrough, Farr & Randall (1951), crystalline bovine albumin (British Drug Houses Ltd.) being used as the standard.

Preparation and extraction of acetone-dried powders. A thick suspension of the bacteria was squirted with rapid stirring into 10 vol. of acetone at either -20° or room temperature (cf. Shukuya & Schwert, 1960). The resulting

precipitate was collected, washed with cold acetone and then ether and drained.

Aqueous suspensions (approx. 5%, w/v) of the acetone-dried powders, immersed in a freezing mixture, were disrupted ultrasonically by a $\frac{3}{4}$ in. probe at 1.3 A for 20 min. The preparations were centrifuged at 11000 rev./min. (11000g) in the MSE Superspeed centrifuge for 30 min., and the yellow supernatant (cell-free extract) was retained.

Glutamate decarboxylase

Precipitation of nucleic acids. The cell-free extract was adjusted to pH 6.0. An aqueous 1% solution of protamine sulphate (pH 2.5) was slowly added with constant stirring, 0.1–0.15 mg. of protamine sulphate being added/mg. of protein. The resultant fine precipitate was removed by centrifugation at 11000 rev./min. (11000g) in the MSE Superspeed centrifuge for 20 min. at 4°.

Fractionation with ammonium sulphate. Solid ammonium sulphate was added to the supernatant from the preceding step to give 26% saturation, the addition being made with constant mechanical stirring. The resulting precipitate was collected by centrifugation and discarded; additional ammonium sulphate was then added to the supernatant to achieve 70% saturation. The precipitate that formed was dissolved in deionized water (2 ml./g. of acetone-dried powder used in the extraction) and insoluble material removed by centrifugation. This preparation was diluted to give a 1% protein solution and incubated at 37° for 1 hr. The precipitate that appeared during this time was removed, the solution was brought to 30% saturation with ammonium sulphate and the resultant supernatant brought up to 65% saturation. The precipitate obtained was dissolved in water and dialysed overnight against 0.1 M-pyridine-pyridine hydrochloride buffer, pH 4.6, at 4°. Any precipitate that appeared was discarded. The solution was then dialysed against 0.04 M-acetate buffer, pH 4.4, at 4° overnight (or for 8 hr.) to remove pyridine. The solution was further dialysed overnight against 0.05 M-sodium phosphate buffer, pH 6.0, before application to a column (1 cm. × 10 cm.) of DEAE-cellulose that had been equilibrated in the same buffer. The protein was eluted from the column with a linear gradient of equal volumes of 0.05 M- and 0.30 M-sodium phosphate buffer, pH 6.0, the eluate being collected in 6 ml. fractions. Those fractions with a specific activity above 10000 μl. of CO₂/mg./10 min. were combined and the solution was brought to 60% saturation with solid ammonium sulphate. The fine suspension was centrifuged at 14000 rev./min. (18000g) in the MSE Superspeed centrifuge for 20 min., and the solid dissolved in a minimum of water. The enzyme was rechromatographed in the same manner except that elution was between 0.05 M- and 0.35 M-sodium phosphate buffer, pH 6.5. Protein estimations were made at various stages throughout the purification.

Specificity of the purified enzyme. The enzyme was tested for activity against six common amino acids and against a number of substances structurally similar to L-glutamic acid.

Optimum pH. This was determined by measurements in the range 2.5–5.5. For pH values up to 3.5, 0.1 M-glycine-glycine hydrochloride buffers were used. For the higher pH values, 0.1 M-acetate buffers were used.

Ultraviolet spectrum. Active fractions from the DEAE-cellulose column were used. Phosphate buffer, pH 6.0

(0.175 M), was used as a blank when fractions from a column were investigated directly.

Addition of pyridoxal phosphate to undialysed enzyme. Chromatographed fractions of the enzyme (final concn. 10 μ g./ml.) were incubated with an aqueous solution of PLP (final concn. 2.5 μ g./ml.) in a Warburg flask for 15 min. at 37°, after which glutamic acid was added and the decarboxylase activity of the system determined. This was repeated with a more concentrated solution of PLP (10 mg./ml.). Suitable controls were also set up. An active rechromatographed fraction was similarly treated.

Addition of pyridoxal phosphate to dialysed enzyme. Portions of a chromatographed enzyme fraction that had been dialysed against a large volume of 0.1 M-acetate buffer, pH 4.8, for 17 hr. or 3 days were used.

Effect of hydroxylamine and semicarbazide on enzyme activity. A solution of the purified enzyme, containing 0.1 mg., was treated with a solution of hydroxylamine or semicarbazide (final concn. 5 mM). The enzyme was incubated with each reagent for approx. 15 min. before the addition of substrate. A dialysed portion of the enzyme was similarly treated.

Resolution of the apoenzyme. To achieve this, the method described by Smith & Waygood (1961) involving rapid precipitation from an ammoniacal solution by the addition of acid was employed. The enzyme was subjected to this treatment only once. The original enzyme solution, the resolved enzyme and the resolved enzyme with PLP added (final concn. 0.25 mg./ml.) were tested for their activities. The controls included one with PLP and glutamic acid in buffer alone.

Arginine decarboxylase

Nucleic acids were precipitated as described for glutamate decarboxylase.

Fractionation with ammonium sulphate. The procedure was again the same as that used in the purification of glutamate decarboxylase, but activity was found in the two precipitates resulting from 26% and 30% saturation. These precipitates were dissolved in water and dialysed as before. The most active fraction was chromatographed on DEAE-cellulose as for the glutamate decarboxylase described above.

Optimum pH. Measurements of activity were made in the range pH 3.4–5.6 in 0.1 M-acetate buffers.

Ultraviolet spectrum. A purified fraction (0.05 mg./ml.) at pH 6.0 was used.

Addition of pyridoxal phosphate to undialysed enzyme. A number of fractions from column chromatography were incubated with PLP (final concn. 0.25 mg./ml.) at 37° for 30 min. before addition of the arginine.

Addition of pyridoxal phosphate to dialysed enzyme. Preparations were subjected to dialysis for 24 hr. against 0.1 M-acetate buffer, pH 4.0, at 4°.

Effect of hydroxylamine and semicarbazide. The activity of the enzyme preparations was measured in the presence of 5 mM-semicarbazide and hydroxylamine.

Histidine decarboxylase

The procedure was essentially that described by Epps (1945), the resulting preparation being dialysed against pyridine-pyridine hydrochloride buffer, pH 4.6, before chromatography on a column (1 cm. $\times$ 7.5 cm.) of DEAE-cellulose set up in 0.05 M-phosphate buffer, pH 6.0, and chilled to 0°. The dialysed enzyme preparation was applied to the column and eluted by phosphate buffer, pH 6.0, of gradually decreasing molarity from 0.3 M to 0.05 M. The eluate was collected in 2.5 ml. fractions.

Specificity. The activity at various stages of purification was determined against a number of amino acids. The pure enzyme was also tested against spinacin, formed from the reaction of formaldehyde with histidine (Neuberger, 1944).

Optimum pH. The enzyme activity was measured in the range pH 3.0–5.3, with glycine and acetate buffers.

Addition of pyridoxal phosphate. A cell-free extract and the final preparation were dialysed against 0.1 M-acetate buffer, pH 4.0, for 24 hr. at 4°, and then treated with PLP (final concn. 0.0625 mg./ml. and 0.1875 mg./ml.).

Addition of hydroxylamine. A dialysed fraction of the pure enzyme was treated with hydroxylamine (final concn. 10 mM).

Resolution of the enzyme. The method of Smith & Waygood (1961) was employed; as with the glutamate decarboxylase, precipitation was carried out once only. The untreated enzyme, the treated enzyme and the latter with PLP added (final concn. 0.25 mg./ml.) were then tested for decarboxylase activity.

RESULTS AND DISCUSSION

Glutamate decarboxylase. Table 1 shows the increase in activity through the various stages of

Table 1. *Glutamate decarboxylase activity at various stages of purification*

Details of purification methods are in the text.

Stage of purification	Total protein (mg.)	Sp. activity (μ l. of CO ₂ /mg./ 10 min.)		Yield (%)
			Q _{CO₂}	
Cell-free extract	40.635	657.5	3945	100
After pptn. with protamine sulphate	38.40	631.5	3789	91
After pptn. with 26% and 70% satd. (NH ₄) ₂ SO ₄	4.515	2571	15426	44
After pptn. with 30% and 65% satd. (NH ₄) ₂ SO ₄	2.35	3606	21636	32
After dialysis	1.045	7043	42258	28
After chromatography	0.2266	13925	83550	12
After rechromatography	0.1530	16871	101226	10

Table 2. *Activities of glutamate decarboxylase, histidine decarboxylase and arginine decarboxylase at various purification stages against a number of substrates*

Details of purification and test methods are in the text. The following abbreviations are used: γ -ABA, γ -amino-*n*-butyric acid; L-ABA, α -amino-*n*-butyric acid; α AisoBA, α -aminoisobutyric acid; n.t., not tested; + + +, extremely active; + +, very active; +, active; $\pm$, of doubtful activity; —, not active.

Preparation	Glu	Asp	Arg	His	Orn	Lys	Glutaric acid	Glu-(NH ₂)	γ -ABA	L-ABA	α -AisoBA	Spin- acin
Glutamate decarboxylase												
Bacteria	+	$\pm$	+	+	+	+	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Acetone-dried powder	++	$\pm$	+	+	—	+	—	—	—	—	—	—
Water extract	++	—	+	—	—	+	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
After 65%-satd. (NH ₄) ₂ SO ₄ fractionation	++	—	—	—	—	—	—	$\pm$	n.t.	n.t.	n.t.	n.t.
After chromatography	+++	—	—	—	—	—	—	$\pm$	n.t.	n.t.	n.t.	n.t.
Histidine decarboxylase												
Bacteria*	+	—	+	+	—	—	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Water extract	—	—	$\pm$	+	—	—	n.t.	n.t.	n.t.	n.t.	n.t.	—
Acetone-dried powder	—	—	$\pm$	+	—	—	n.t.	n.t.	n.t.	n.t.	n.t.	—
After chromatography	—	—	—	+	—	—	n.t.	n.t.	n.t.	n.t.	n.t.	—
Arginine decarboxylase												
Bacteria*	—	—	+	—	—	—	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
After chromatography	—	—	+++	—	—	—	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.

* These bacteria were grown on supplemented minimal media instead of standard nutrient broth.

Table 3. *Effect of addition of pyridoxal phosphate on decarboxylase activity (Q_{CO_2})*

Details of purification and test methods are in the text.

	Q_{CO_2}			
	Enzyme prep. alone	With PLP (2.5 μ g./ml.)	With PLP (10 μ g./ml.)	With PLP (62.5 μ g./ml.)
Glutamate decarboxylase				
Chromatographed	16756.8	—	18426	—
Rechromatographed	22500	29850	—	—
Arginine decarboxylase				
Chromatographed {	6588	6948	—	—
	8100	11340	—	—
	5040	9660	—	—
Histidine decarboxylase				
Chromatographed	376.0	—	—	386.0

purification. The most active fraction obtained compares favourably with preparations obtained by Shukuya & Schwert (1960) and Melius (1966), and the results indicate that the enzyme is PLP-dependent. Table 3 shows that PLP (10 μ g./ml.) did enhance the activity of a chromatographed fraction, indicating that some loss of cofactor had

occurred. Rechromatography had a marked effect on cofactor removal. This suggests that there is a tight binding of the cofactor, a view that is confirmed by the results of dialysis (Table 4), which decreased the enzyme activity by 25 and 40% after dialysis for 17 hr. and 3 days respectively. In both cases over 80% of the original activity was restored

Table 4. *Effect of addition of pyridoxal phosphate on the activity of dialysed enzyme preparations (Q_{CO_2})*

Details of test methods are in the text.

	Q_{CO_2}			
	Enzyme alone	Enzyme dialysed for 17 hr.	Enzyme dialysed for 3 days	Dialysed enzyme + PLP (10 μ g./ml.)
Glutamate decarboxylase	14 720	{ 11 560 —	— 6 347	13 944 11 495
Arginine decarboxylase	4 017	3 908	—	3 816

Table 5. *Effect of semicarbazide and hydroxylamine on enzyme activity (Q_{CO_2})*

Details of test methods are in the text.

	Q_{CO_2}		
	Enzyme alone	With semi-carbazide (5 mm)	With hydroxylamine (5 mm)
Glutamate decarboxylase	7 406	7 179	0
Glutamate decarboxylase (dialysed prep.)	6 347	5 597	0
Arginine decarboxylase	{ 10 320 7 200	8 908 6 320	8 720 7 810
Histidine decarboxylase	106.7	—	109.9*

* In this case the hydroxylamine concentration was 10 mm.

Table 6. *Effect of resolution procedure on enzymic activity (Q_{CO_2}) and of addition of PLP to the resulting preparations*

Details of test methods are in the text.

	Q_{CO_2}		
	Original enzyme solution	Treated enzyme	Treated enzyme + PLP (0.25 mg./ml.)
Glutamate decarboxylase	10 109	210.0	2 318
Histidine decarboxylase	290.4	63.7	115.8

Fig. 1. pH-activity curves of (a) glutamate decarboxylase, (b) arginine decarboxylase and (c) histidine decarboxylase. ●, In glycine-glycine hydrochloride buffer; ○, in acetate buffer.

Fig. 2. Ultraviolet spectrum of glutamate decarboxylase at various pH values. A 0.2mg./ml. solution of the enzyme was used.

Fig. 3. Stability of glutamate decarboxylase at different temperatures. The enzyme preparation (2.5ml. of a solution containing 0.3 mg. of protein/ml.) was held for 1 hr. at each temperature indicated before the activity was determined. $\blacktriangle$, 17°; $\bullet$, 30°; $\blacksquare$, 37°; $\circ$, 48.5°; $\triangle$, 60°; $\square$, 70°.

by addition of PLP. Semicarbazide, in contrast with hydroxylamine, only became effective as an inhibitor when loss of cofactor had occurred (Table 5). Failure to reconstitute the enzyme completely after resolution by addition of PLP (see Table 6) may be due to insufficient incubation of the apo-enzyme with the PLP.

The enzyme preparations had no activity against any of a number of amino acids used, after the first fractionation with ammonium sulphate (Table 2). After chromatography, only glutamic acid and glutamine ($Q_{CO_2} < 20$) were decarboxylated. As Roberts & Frankel (1951) have suggested, decarboxylation of glutamine may be due not to lack of specificity but to the presence of glutaminase, which would liberate glutamic acid, or to the presence of traces of a distinct L-glutamine decarboxylase.

The enzyme showed optimum activity at pH 3.65 (Fig. 1a), a value in close agreement with that of Shukuya & Schwert (1960), who used pyridine-pyridine hydrochloride buffer and suggested that the higher values obtained by other workers with acetate buffer might be due to the blocking of the enzyme surface by un-ionized acetic acid. Our result was, however, obtained with acetate buffer.

The u.v. spectrum of the enzyme (Fig. 2) shows that a pH change from 4.0 to 7.5 is accompanied by the disappearance of a maximum at 410mμ and an increase in the absorption at 340–360mμ. As it has been established that the enzyme has optimum activity at about pH 4, it seems that this absorption

Table 7. *Histidine decarboxylase activity at various stages of purification*

Details of purification methods are in the text.

Stage of purification	Total protein (mg.)	Sp. activity (μ l. of CO ₂ /mg./ 10 min.)	Q _{co₂}	Yield (%)
Water extract	10.8	6.06	36.4	100
After pptn. with protamine sulphate	7.64	8.19	49.16	94
Eluate from gel	1.11	24.20	145.2	41
Solution of ppt. from pptn. with satd. (NH ₄) ₂ SO ₄	0.96	26.10	156.9	36
After dialysis	0.56	48.20	289.0	38
After chromatography (most active fraction)	0.216	78.3	478.0	26

Table 8. *Arginine decarboxylase activity at various stages of purification*

Details of test methods are in the text.

Stage of purification	Total protein (mg.)	Sp. activity (μ l. of CO ₂ /mg./ 10 min.)	Q _{co₂}	Yield (%)
Water extract	34.10	492.6	2976	100
After pptn. with protamine sulphate	24.2	644.5	3867	93
Solution of ppt. from 26%-satd. (NH ₄) ₂ SO ₄ (i)	1.41	904.0	5424	8
(i) After dialysis	1.10	1062	6374	7
Solution of ppt. from 30%-satd. (NH ₄) ₂ SO ₄ (ii)	1.33	1925	11750	15
(ii) After dialysis	0.95	2350	14100	13
After chromatography	0.76	2700	16200	12

Fig. 4. Ultraviolet spectrum of arginine decarboxylase at pH 6.0. A 0.05 mg./ml. solution of the enzyme was used.

at 410 m μ is characteristic of the active enzyme. The enzyme shows unusual stability, being little affected by storage at 0°. Fig. 3 shows the effect

of temperature on activity after a sample had stood for 1 hr. at pH 4.4.

Histidine decarboxylase. The activity of processed preparations of this enzyme (Table 7) was disappointing as compared with that of preparations of glutamate decarboxylase. The small increase in activity (6.0%) of dialysed fractions on addition of PLP (final concn. 0.19 mg./ml.) and the absence from these fractions of any inhibition by hydroxylamine (Table 5) would indicate that the coenzyme is tightly bound. Partial resolution of the enzyme was, however, achieved (Table 6) as with glutamate decarboxylase, and it therefore appears that, like other decarboxylases, this one is PLP-dependent.

Purification of the enzyme removed activity towards arginine, which apart from histidine was the only amino acid of a number tested to be attacked by the crude preparation. Spinacin, a formaldehyde derivative of histidine (Neuberger, 1944), was not attacked, a finding that might suggest that the amino group is directly involved in a Schiff base complex.

The pH optimum of histidine decarboxylase was 4.52 (Fig. 1c), a value in agreement with that of Gale (1946), below that of Eggleston (1958) and within the range quoted by Rosenthaler *et al.* (1965).

Arginine decarboxylase. The procedure employed

for the purification of this enzyme (Table 8) was identical with that used for glutamate decarboxylase except that the temperature was kept at 0°. The pH optimum was found to be 4.9 (Fig. 1b), a value intermediate between Gale's (1940) value of 4.0 for a washed bacterial suspension, and the 5.25 of Taylor & Gale (1945) for a purified extract. The latter value was also obtained by Leclerc, Osteux & Guillaume (1963) using an enzyme preparation from *Aeromonas shigelloides*.

The purified enzyme was inactive against any of the common amino acids except arginine, and its u.v. spectrum showed marked absorption at 250–260 m μ (Fig. 4). Table 3 shows the enhancing effect of PLP addition on the activity of chromatographed fractions, and these results suggest that the enzyme is PLP-dependent. Dialysis failed to remove any coenzyme from these preparations, again indicating a tight bonding between apo- and co-enzyme.

At a concentration of 0.01 M, semicarbazide and hydroxylamine had little effect on enzyme activity, but at higher concentrations inhibition occurred with the former and enhancement with the latter. These findings lend support to those of Gonnard (1962) and Makino *et al.* (1962), who claimed that derivatives of PLP formed by condensation with carbonyl reagents could act as coenzyme in certain pyridoxal-dependent systems.

REFERENCES

- Aiso, K., Yanagisawa, F., Toyoura, H., Fujita, H. & Toyoura, K. (1955). *Sogo Igaku*, **12**, 111; cited in *Chem. Abstr.* (1960) **54**, 16543.
- Bonavita, V. & Scardi, V. (1958). *Experientia*, **14**, 133.
- Cozzani, I. (1965). *G. Biochim.* **14**, 665.
- Davis, B. D. & Mingioli, E. S. (1950). *J. Bact.* **60**, 17.
- Eggleston, L. V. (1958). *Biochem. J.* **68**, 557.
- Ekladius, L., King, H. K. & Sutton, C. R. (1957). *J. gen. Microbiol.* **17**, 602.
- Epps, H. M. R. (1945). *Biochem. J.* **39**, 42.
- Gale, E. F. (1940). *Biochem. J.* **34**, 392.
- Gale, E. F. (1946). *Advanc. Enzymol.* **6**, 1.
- Ganrot, P. O., Rosengren, A. M. & Rosengren, E. (1961). *Experientia*, **17**, 263.
- Gonnard, P. (1962). *I.U.B. Symp. Ser.* **30**, 307.
- Guirard, B. M. & Snell, E. E. (1954). *J. Amer. chem. Soc.* **76**, 4745.
- Haughton, B. G. & King, H. K. (1961). *Biochem. J.* **80**, 268.
- Kornberg, H. L., Phizackerly, P. J. R. & Sadler, J. R. (1960). *Biochem. J.* **77**, 438.
- Leclerc, H., Osteux, R. & Guillaume, J. (1963). *Ann. Inst. Pasteur*, **104**, 477.
- Linstedt, S. (1951). *Acta chem. scand.* **5**, 486.
- Lovenberg, W., Weissbach, H. & Udenfriend, S. (1962). *J. biol. Chem.* **237**, 89.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Makino, K., Ooi, Y., Matsuda, M. & Kuroda, T. (1962). *I.U.B. Symp. Ser.* **30**, 291.
- Mardashev, S. R. & Semina, L. A. (1964). *Dokl. Akad. Nauk S.S.S.R.* **156**, 465.
- Melius, P. (1966). *Canad. J. Biochem.* **44**, 145.
- Najjar, V. A. & Fisher, J. (1954). *J. biol. Chem.* **206**, 215.
- Neuberger, A. (1944). *Biochem. J.* **38**, 309.
- Ono, S. & Hagen, P. (1959). *Nature, Lond.*, **184**, 1143.
- Roberts, E. & Frankel, S. (1951). *J. biol. Chem.* **188**, 789.
- Rodwell, A. W. (1953). *J. gen. Microbiol.* **8**, 233.
- Rosenthaler, J., Guirard, B. M., Chang, G. W. & Snell, E. E. (1965). *Proc. nat. Acad. Sci., Wash.*, **54**, 152.
- Schayer, R. W. & Sestokas, E. (1965). *Biochim. biophys. Acta*, **111**, 557.
- Shukuya, R. & Schwert, G. W. (1960). *J. biol. Chem.* **235**, 1649, 1653.
- Smith, J. E. & Waygood, E. R. (1961). *Canad. J. Biochem. Physiol.* **39**, 1055.
- Taylor, E. S. & Gale, E. F. (1945). *Biochem. J.* **39**, 52.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1961). *Manometric Techniques and Tissue Metabolism*, 2nd ed., pp. 1–17, 218. Minneapolis: Burgess Publishing Co.
- Umbreit, W. W. & Gunsalus, I. C. (1945). *J. biol. Chem.* **159**, 333.
- Umbreit, W. W. & Heneage, P. (1953). *J. biol. Chem.* **201**, 15.