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The Histidine Decarboxylase of a Species of *Lactobacillus*; Apparent Dispensability of Pyridoxal Phosphate as Co-enzyme

By A. W. RODWELL*

*Medical Research Council Unit for Chemical Microbiology, Biochemical
Laboratory, University of Cambridge*

SUMMARY: The histidine decarboxylase produced by a species of *Lactobacillus* (strain 30a isolated from horse-stomach contents; Rodwell, 1953a) has been examined in detail. Attempts to resolve a cell-free enzyme preparation into an inactive apoenzyme and a coenzyme were unsuccessful. Cells grown in a medium deficient in pyridoxin had greatly diminished lysine and ornithine decarboxylase activity, whereas their histidine decarboxylase activity was not at all diminished.

The four bacterial enzymes which specifically decarboxylate the L-isomers of arginine, lysine, ornithine and tyrosine respectively have been shown to require a phosphorylated form of pyridoxal as coenzyme (Gunsalus, Bellamy & Umbreit, 1944; Baddiley & Gale, 1945). The enzymes which attack glutamic acid and histidine have resisted all attempts to resolve them into apoenzyme and coenzyme moieties (Taylor & Gale, 1945; Epps, 1945), and codecarboxylase was not released by boiling histidine and glutamic acid decarboxylase preparations (Epps, 1945). The four enzymes which are known to require pyridoxal phosphate as coenzyme were found to be relatively more sensitive to inhibition by keto (or aldehyde) fixatives such as phenylhydrazine or semicarbazide than were the glutamic acid or histidine decarboxylases (Taylor & Gale, 1945). These findings led Gale (1946) to express the view that the bacterial glutamic acid and histidine decarboxylases do not require pyridoxal phosphate as coenzyme. Umbreit & Gunsalus (1945), however, have described one experiment in which the activity of glutamic acid decarboxylase which was lost after dialysis in the cold at pH 2 was partially restored by the addition of codecarboxylase to the system. Werle & Koch (1949) claim to have shown an activation by pyridoxal phosphate of histidine decarboxylase preparations from both guinea-pig tissue and *Bacterium coli*. Gunsalus (1950) in a review stated that pyridoxal phosphate has now been shown to be the coenzyme for all the amino-acid decarboxylases, whether of bacterial, plant or tissue origin.

However, in the experiments reported in this paper, no evidence was obtained that the histidine decarboxylase produced by a strain of *Lactobacillus* requires codecarboxylase, and it is suggested that more rigid proof is necessary before histidine decarboxylase is included among the enzymes which require pyridoxal phosphate.

* Present address: Council for Scientific & Industrial Research Organization, Animal Health Research Laboratory, Parkville, Melbourne, N.2, Australia.

METHODS

Organism and media. The strain of *Lactobacillus* used, strain 30*a*, and the composition of the semi-defined medium used in the pyridoxin deficiency experiments were described in a previous paper (Rodwell, 1953*a*).

Determination of decarboxylase activity. This was determined manometrically at a temperature of 30°; the pH value was controlled with McIlvaine buffer and oxygen-free nitrogen was the gas phase. Decarboxylase activities of washed suspensions are expressed as Q_{CO_2} . The notation $Q_{\text{CO}_2}(E)$ is used to express the activities of the cell-free enzyme, and is defined as the $\mu\text{l. CO}_2$ evolved/hr. by the extract from 1 mg. acetone powder (which corresponds almost exactly to the enzyme obtained from 1 mg. dry wt. of cell suspension).

Preparation of cell-free histidine decarboxylase. The acetone method described by Gale & Epps (1943) for making cell-free preparations of bacterial decarboxylases was found to be very suitable for the preparation of the histidine decarboxylase of strain 30*a* in the cell-free condition. The optimum pH value for the extraction of enzyme from the acetone-dried powder was from 4.8 to 5.2. Best extraction was obtained by incubating the powder suspended in McIlvaine buffer at pH 4.8 for 8 hr. at 37°. A slightly yellow, sparkling clear extract was obtained after centrifugation. Extract prepared in this way had, in one experiment, an activity expressed as $Q_{\text{CO}_2}(E)$ of 480, whereas the activity of the washed suspension used in its preparation had a Q_{CO_2} of 320.

Optimum pH values for the activity of the enzymes. The optimum pH values for the decarboxylation of histidine, ornithine and lysine by washed suspensions were determined in McIlvaine buffer at 4.0, 5.5 and 6.0 respectively. The optimum pH for the cell-free histidine decarboxylase was at pH 4.8.

Preparation of buffer solutions used for dialysis of histidine decarboxylase. Buffer solutions at pH values 2 and 4 were prepared by titrating 0.01M solutions of sodium acetate containing 0.05M-sodium chloride with N-hydrochloric acid by means of a glass electrode; buffer solution at pH 6.0 by titrating 0.01M-citric acid containing 0.05M-sodium chloride with N-sodium hydroxide; and buffer solution at pH 8.6 by titrating 0.01M-boric acid solution containing 0.05M-sodium chloride with N-sodium hydroxide.

Attempts to resolve histidine decarboxylase

Attempts were made to resolve the cell-free histidine decarboxylase prepared from strain 30*a* by dialysis at 37° against the buffer solutions described above, and also by dialysing the enzyme solution alternately against saturated ammonium sulphate solution made alkaline with ammonia, and against tap water.

Dialysis against dilute buffer solutions. Four portions, each of 10 ml., of the acetone-powder extract were dialysed in cellophan sacs against running tap water for 3 hr., then for 5 hr. in various buffer solutions. The buffer solutions were contained in measuring cylinders (1 l.) standing in a tank of water at 37°, and the buffer solutions (previously warmed to 37°) were changed twice during the 5 hr. period. The extracts were then dialysed against running tap

water for 12 hr. The histidine decarboxylase activity of the undialysed extract, and of the extracts after dialysis, was determined on suitable samples in presence or absence of a boiled, washed suspension of cells of strain 30*a*; the results are shown in Table 1.

Table 1. *Effect of dialysis against dilute buffer solutions on activity of the cell-free histidine decarboxylase of strain 30a*

Cell-free extracts dialysed against 0.1M buffer solutions at 37°. Activity of dialysed extracts tested in the presence (+) or absence (–) of a boiled aqueous suspension of cells of strain 30*a*, or of a boiled aqueous acetone powder suspension. Manometer flasks contained extract equivalent to 1 mg. acetone powder and boiled cell or acetone powder extract equivalent to 5 mg. dry wt. Activities are expressed as percentages of the original activity of the undialysed extract, after correction for volume changes during dialysis.

Dialysis at 37° for				
5 hr.				23 hr.
pH value of dialysis				
2.0	4.0	6.0	8.6	8.6
Activity after dialysis as % original activity				
– boiled cells				
6.4	72	80	94	43
+ boiled cells				
6.9	73	83	96	43

The activity of the extracts after dialysis progressively decreased as the pH value of the mixture being dialysed was lowered from 8.6 to 2.0; in no case was a significant reactivation obtained by testing the dialysed extracts in the presence of boiled cell suspension. In another attempt, extract was dialysed against three changes of buffer at pH 8.6 for a total of 23 hr. at 37°. The dialysed extract was tested in the presence and absence of a boiled aqueous suspension of the acetone powder of strain 30*a*. The activity after dialysis had decreased to 43 % of that of the original extract, but no reactivation was obtained by the addition of boiled acetone powder suspension (Table 1).

Dialysis against ammoniacal ammonium sulphate solution. Acetone-powder extract was subjected to the following successive treatments: (i) dialysis against saturated ammonium sulphate solution (containing 3 % (v/v) of 0.880 sp. gr. ammonium hydroxide solution) for 2 hr. at room temperature; (ii) dialysis against running tap water for 1 hr.; (iii) dialysis against ammoniacal ammonium sulphate for another 2 hr. period; (iv) against tap water for 12 hr.

The protein precipitated by dialysis against the ammoniacal ammonium sulphate solution was completely redissolved after both subsequent dialyses against tap water. The histidine decarboxylase activity of the dialysed, and of the original undialysed extract, was tested both with and without the addition of a boiled aqueous suspension of cells of strain 30*a*. The manometer flasks contained extract equivalent to 1 mg. acetone powder, and boiled suspension containing 5 mg. dry wt. of cells. The $Q_{\text{co}_2}(E)$ values found (after correction for volume changes during dialysis) were 440 for the undialysed

suspension both with and without boiled cells, and 286 and 283 for the dialysed extract tested in the presence and absence of boiled suspension respectively.

*Effect of a pyridoxin deficiency in the growth medium on
the activity of the decarboxylases*

Since all attempts to split the histidine decarboxylase of strain 30*a* into an inactive apoenzyme and a coenzyme were unsuccessful, the deficient culture technique was applied to the problem. Strain 30*a* has an absolute requirement for pyridoxin (Rodwell, 1953*a*). The results obtained in two experiments are set out in Table 2. The cells were grown in the semi-defined medium modified by adjusting the pyridoxin content as indicated (Table 2). In both experiments histidine decarboxylase activity was higher in cells harvested from medium deficient in pyridoxin, whereas the ornithine and lysine decarboxylase activities of the cells were very markedly decreased. In these particular experiments only a slight degree of activation of the apoenzymes of ornithine and lysine decarboxylases was obtained by testing pyridoxin-deficient cells in the presence of pyridoxal. However, it was later found that the concentrations of pyridoxal and the time of incubation used in these tests were insufficient to obtain appreciable activation of the apoenzymes in washed cell suspensions of this organism. The activation of ornithine apodecarboxylase in pyridoxin-deficient cells of strain 30*a* by pyridoxal is described elsewhere (Rodwell, 1953*b*). The results obtained in one experiment may be anticipated: the rate for the decarboxylation of ornithine by a pyridoxin-deficient cell suspension was $Q_{\text{CO}_2} = 5$ in the absence of added pyridoxal, but the rate when the cells were tested in the presence of 0.001M-pyridoxal increased to a maximum value of $Q_{\text{CO}_2} = 1100$. No increase in the rate for the decarboxylation of histidine was ever found when pyridoxin-deficient cells were tested with similar pyridoxal concentrations.

Table 2. *Effect of pyridoxin deficiency in the growth medium on the ornithine, lysine and histidine decarboxylases of Lactobacillus sp. strain 30a*

Cells were grown in semi-defined medium modified by adjusting the pyridoxin content as indicated.

Pyridoxin added to medium ($\mu\text{g./ml.}$)	Decarboxylation of		
	Histidine	Ornithine Q_{CO_2} values	Lysine
10.00	130	116	nt
0.10	232	7	nt
None	185	4	nt
10.00	155	160	220
0.05	264	8	5
None	355	1	2

nt = no test.

DISCUSSION

Failure to resolve the cell-free histidine decarboxylase preparation by the rather severe treatments used may imply a very high affinity of apoenzyme for coenzyme. It would then be expected that a deficiency of pyridoxin in the

growth medium would depress the ornithine and lysine decarboxylase activities of the cells before histidine decarboxylase was affected. The latter might only be affected by extreme deficiency, perhaps at concentrations of pyridoxin too low to allow appreciable growth. In these experiments growth in the medium to which no pyridoxin had been added was approximately half that obtained in the media containing pyridoxin. The production of cells extremely deficient in pyridoxin derivatives by replacing pyridoxin in the growth medium by D-alanine was not tried. These experiments therefore do not prove that the coenzyme for histidine decarboxylase is not pyridoxal phosphate. Nevertheless, when these results are considered together with the earlier work of Epps (1945), and of Taylor & Gale (1945), it is felt that the evidence strongly suggests that histidine decarboxylase does not require pyridoxal phosphate.

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