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EDWARD C. HORN*

Biology Division, Oak Ridge National Laboratory,
Oak Ridge, Tennessee (U.S.A.)

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* On sabbatical leave from the Department of Zoology, Duke University, Durham, North Carolina.

Diaminopimelic acid decarboxylase in pyridoxin-deficient *Escherichia coli*

The bacterial amino acid, diaminopimelic acid (DAP) is decarboxylated to L-lysine by a specific enzyme (DAP decarboxylase) which occurs in many bacteria[1,2,3,4]. This enzyme differs from the majority of the bacterial amino acid decarboxylases in that it is a constitutive enzyme functioning at physiological pH, but it resembles them in its activation by pyridoxal phosphate. In *Escherichia coli*, the function of DAP decarboxylase is probably to provide a biosynthetic route to lysine[5].

Using a pyridoxin-requiring mutant of *E. coli* (derived from 154-59L), a study has been made of the effects of pyridoxin deficiency on the cellular levels of DAP decarboxylase and also of the adaptive lysine decarboxylase. Pyridoxin deficiency was produced by growing the mutant on a simple medium (DAVIS AND MINGIOLI[6] with 0.5% glucose and 0.45% citrate) containing the minimal level of pyridoxin (20 μg/l) necessary for growth. Control cells were grown on a pyridoxin level of 500 μg/l. These media were used both with and without supplements of L-lysine (200 mg/l). A 24 h culture in 100 ml of medium containing 2 μg pyridoxin was used as an inoculum for 2 l batches of medium; growth was continued for 24 h at 37° in the dark with vigorous aeration. The cells were harvested by centrifuging, washed and acetone-dried. DAP and lysine decarboxylases were measured at pH 6.8 in the Warburg apparatus both with and without added pyridoxal phosphate. pH 6.8 was chosen as being near the optimum for DAP decarboxylase in *E. coli*[1]; this is not the optimum for lysine decarboxylase, the values for which were about twice as high at pH 5.0 as at pH 6.8. Since lysine decarboxylase, when present, is operative in the measurement of DAP decarboxylase[3], the values for both enzymes are reported at the same pH.

Vitamin B_6 assays were carried out microbiologically by a method modified somewhat from that of ATKIN *et al.*[7]. The test organism was a variant of *Saccharomyces carlsbergensis* 4228 which required pyridoxin absolutely for growth even in the absence of thiamine. The basal medium contained less casein hydrolysate (0.2% final) and the cultures were sloped (not shaken) during a 22 h incubation. The samples were extracted with 45 ml 0.055 *N* H_2SO_4 at 121° for 1 h before assaying the supernatant. The method assays equally all known forms of vitamin B_6 and the results are expressed in terms of pyridoxin.

The results (see Table) show that the cells grown on minimal pyridoxin contained only about 11% of the pyridoxin of the control cells. The presence of lysine in the growth medium apparently had a pyridoxin-sparing effect, since in both deficient and control cells the lysine-grown cells had a higher pyridoxin content. The possibility of contamination of the lysine by traces of pyridoxin was not investigated, but it seemed unlikely in view of the similarity of the results obtained with high and low pyridoxin levels. Addition of pyridoxal phosphate to the Warburg flasks caused stimulation of decarboxylase activity in pyridoxin-deficient cells, but had little effect on the control cells, showing that a genuine state of pyridoxin deficiency had been produced. Lysine decarboxylase was absent from both cultures grown without additional lysine. The apoenzyme of DAP decarboxylase (estimated in the presence of pyridoxal phosphate) was about twice as high in the deficient cells grown without lysine as it was in the control cells or in the deficient cells grown with lysine. On the other hand, the coenzyme-bound DAP decarboxylase (holoenzyme, estimated without additional pyridoxal phosphate) was markedly reduced in the deficient cells grown in the presence of lysine.

These results can be explained by assuming that the main function of DAP decarboxylase is to provide lysine for the cell. Under the condition of stress caused by deficiency of both pyridoxin and lysine, the amount of DAP decarboxylase apoenzyme is raised, so keeping the coenzyme-bound enzyme at its normal level in spite of scarcity of pyridoxin. When the pyridoxin-deficient cells are provided with exogenous lysine, the need for DAP decarboxylase is not so acute; the apoenzyme remains at its normal level, while the holoenzyme level is depressed thus releasing coenzyme for other essential functions. The reason for the raised level of lysine decarboxylase apoenzyme in these cells is not apparent; this enzyme is a non-essential adaptive enzyme[8]. A considerable rise in histidine decarboxylase holoenzyme has been reported in a Lactobacillus subjected to a moderate degree of pyridoxin deficiency which caused a complete disappearance of ornithine decarboxylase holoenzyme[9]. In a pyridoxin-requiring mutant of *E. coli* (154–59L), lack of pyridoxin produced a subnormal level of D-serine dehydrase holoenzyme, but a lightly raised level of the apoenzyme[10].

An attempt was made to find a pyridoxin-free medium suitable for growth of our mutant, in accordance with the findings of SNELL and collaborators that a mixture of amino acids (including D-alanine) and vitamins could replace pyridoxin[10,11]. The attempt was unsuccessful owing to reversion during growth.

TABLE I

PYRIDOXIN AND DECARBOXYLASE CONTENTS OF ACETONE-DRIED MUTANT *E. coli* GROWN IN DIFFERENT CONCENTRATIONS OF PYRIDOXIN

Enzyme tests carried out in air at 37° on 20–40 mg of cells suspended in 2.5 ml 0.1 *M* phosphate buffer pH 6.8. 0.1 ml of barium pyridoxal phosphate (B6-Phos) solution (0.1 mg/ml) added where shown. Q_{CO_2} = μl CO_2/h/mg cells.

Supplement to minimal medium		*Cellular pyridoxin content* (μg/g)	*Decarboxylase activity* (Q_{CO_2})			
			Diaminopimelic		*Lysine*	
Pyridoxin (μg/l)	*Lysine* (mg/l)		*Alone*	*+ B_6-Phos*	*Alone*	*+ B_6-Phos*
20	0	8.5	6.1	10.1	0	0
20	200	13.6	2.2	5.7	9.8	28.8
500	0	75.6	5.0	5.6	0	0
500	200	85.7	4.4	5.9	17	18

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R. F. DENMAN
D. S. HOARE
ELIZABETH WORK

University College Hospital Medical School, London (England)

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