The Regulation of Diaminopimelate Decarboxylase Activity in *Escherichia coli* strain w

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SUMMARY

Activity of diaminopimelate decarboxylase in *Escherichia coli* strain w, growing in an aerated fermenter, was only slightly (14 %) repressed by 2 mM-lysine when approximately equimolar diaminopimelate was present in the medium. Lysine alone caused 78 % repression. Diaminopimelate did not interfere with uptake of lysine by growing organisms. Organisms grown in medium containing diaminopimelate, without lysine, had a decarboxylase activity 24 % higher than organisms from minimal medium. The extent of repression by pyridoxine (56 % when added to minimal medium) was decreased (to 31 %) when diaminopimelate was also present in the medium. A diaminopimelate-requiring mutant, with limited ability to take up diaminopimelate, formed almost three times less diaminopimelate decarboxylase than did a diaminopimelate-requiring second-stage mutant that had an increased rate of transport of this amino acid. The internal concentration of diaminopimelate thus probably regulates the activity of the decarboxylase by induction. Lysine might not directly repress the enzyme but might give an apparent repression by restricting the biosynthesis of diaminopimelate. This restriction is probably not caused only by inhibition or repression of aspartokinase. Lysine and threonine together, though not singly, almost completely inhibited aspartokinase *in vitro* but caused less apparent repression of diaminopimelate decarboxylase than did lysine alone. Lysine plus diaminopimelate strongly repressed the lysine-sensitive aspartokinase (85 %) without much affecting diaminopimelate decarboxylase formation, and pyridoxine repressed the decarboxylase without affecting aspartokinase.

INTRODUCTION

Diaminopimelate decarboxylase (EC. 4.1.1.20; *meso*-2,6-diaminopimelate carboxy-lyase) catalyses the removal of carbon dioxide from *meso*-diaminopimelate to yield L-lysine (Work, 1962). Pyridoxal phosphate is required as cofactor (White & Kelly, 1965). Enzyme activity in most bacteria is lower when lysine is added to chemically defined media (Denman, Hoare & Work, 1955; Patte, Loviny & Cohen, 1962; White *et al.*, 1964; Gilboe, Friede & Henderson, 1968; Grandgenett & Stahly, 1971). Adding diaminopimelate to minimal media does not significantly alter enzymic activity (White *et al.*, 1964). Hence, diaminopimelate decarboxylase was thought not to be induced by its substrate, but to be repressed by lysine, the end-product of its action. Lysine can also inhibit diaminopimelate decarboxylase action (White & Kelly, 1965; Rosner, 1975). In wild-type *Escherichia coli* strain w and in some mutants of this and of other strains of *E. coli*, the activity of diaminopimelate decarboxylase is also decreased when pyridoxine is present in the growth medium (Denman *et al.*, 1955; White *et al.*, 1964; Bukhari & Taylor, 1971).

The findings reported in this paper suggest that in *E. coli* w the enzymic activity is really controlled by the internal concentration of diaminopimelate, and that the effects of both

lysine and pyridoxine arise by changing the internal concentration of diaminopimelate. A preliminary report of this work has already been made (White, 1974).

METHODS

Organisms. *Escherichia coli* W (ATCC9637) was maintained as described previously (White & Kelly, 1965). Mutant 173-25 (Davis, 1952) is derived from strain W, and requires diaminopimelate plus lysine for rapid growth on a minimal medium, though the mutant will grow slowly in medium supplemented only with diaminopimelate. Mutant D3 was obtained by serial transfers of mutant 173-25 in medium containing diaminopimelate plus decreasing concentrations of lysine, as described by Meadow, Hoare & Work (1957), until organisms were obtained that grew in medium supplemented only with diaminopimelate as rapidly as the wild type grew in minimal medium. The similar mutants D (Meadow *et al.*, 1957) and D2 (Leive & Davis, 1965) were no longer available. Mutant 173-25 was maintained in the same way as the parent strain, but on slopes of nutrient agar plus diaminopimelate (100 mg l⁻¹); minimal medium plus diaminopimelate (100 mg l⁻¹) was used to maintain mutant D3.

Medium. The minimal medium of Davis & Mingioli (1950) was used with 0.2% glucose (autoclaved separately). Additions to the medium are given in Results.

Growth of cultures. Organisms were grown in shaken 2 l flasks containing 250 ml medium, as described previously (White & Kelly, 1965). Larger cultures (11 l) were grown in a Microferm Laboratory Fermenter (New Brunswick Scientific Co., New Brunswick, New Jersey, U.S.A.) at 37 °C with an airflow of 2 l min⁻¹; no antifoam agent was added. The inoculum for the fermenter was approx. 2×10^8 organisms from a fresh slope culture; the organisms were suspended in water and added to the warmed medium. Turbidity became visible in the culture after incubation for about 16 h except when mutant 173-25 was grown without lysine; in this case there was a lag of 24 h before turbidity was visible.

Assay of diaminopimelate decarboxylase. Organisms were harvested and washed, extracts were prepared and enzymic activity was measured manometrically under N₂ as described by White (1971). No correction was made for retention of CO₂ by the buffer (pH 6.8) and all enzymic activities are expressed as manometric units (White & Kelly, 1965).

Assay of lysine decarboxylase. Extracts (about 3 mg protein) plus pyridoxal phosphate (100 µg) and 2-mercaptopropan-1-ol (final concentration 1 mM) were incubated in 0.05 M-phosphate buffer pH 6.0 or 6.8 (2.5 ml). Release of CO₂ from L-lysine (2 mg) was measured manometrically in N₂ at 37 °C in a Warburg apparatus. One unit of lysine decarboxylase was the amount of enzyme that liberated 1 µmol CO₂ in 1 min at pH 6.0.

Assay of aspartokinase. The method of Black & Wright (1955) as described by Truffa-Bachi & Cohen (1970) was used, but with the FeCl₃·6H₂O dissolved in 1 M-HCl. Samples were assayed for product formed after 0, 10, 20 and 30 min, and the rate of reaction was determined graphically. One unit of aspartokinase was the amount of enzyme that formed 1 µmol aspartic acid β-hydroxamate in 1 min.

Estimations. Diaminopimelate was assayed by the acidic ninhydrin method at 37 °C (Work, 1957), in which lysine does not interfere.

Lysine was assayed (in the presence of diaminopimelate) by the method of Shimura & Vogel (1966).

Protein was assayed by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin as standard.

Dry weight of bacterial suspensions was determined as described by White & Kelly (1965).

Growth was measured turbidimetrically with an EEL colorimeter as described by White *et al.* (1964). A colorimeter reading of 1.0 corresponded to 0.3 mg dry wt organisms ml⁻¹ and to 1×10^9 organisms ml⁻¹.

Uptake of [1-¹⁴C]diaminopimelic acid. The procedure was based on the method of Leive & Davis (1965). Organisms were harvested in the exponential phase of growth and washed once in minimal medium without glucose and ammonium salts (solution D). The washed bacteria were suspended in minimal medium with glucose but without ammonium salts, to a density of 0.2 mg dry wt ml⁻¹. This suspension (10 ml) was equilibrated at 37 °C in a test-tube, and at zero time 0.2 ml [1-¹⁴C]diaminopimelate (75 nmol; 2.3×10^4 c.p.m.) in solution D was added. Samples (2 ml at zero time and 5 min, and thereafter 1 ml) were removed, quickly added to 2 ml 0.2 mM-*meso*-diaminopimelate at 0 °C in solution D, and rapidly filtered through Millipore type HA membranes (25 mm diam., 0.45 µm porosity, pre-soaked in 0.2 mM-*meso*-diaminopimelate in solution D). The discs and collected organisms were washed once at room temperature with 3 ml 0.2 mM-*meso*-diaminopimelate in solution D and then transferred to 10 ml scintillation fluid (Bray, 1960) in glass vials. The vials were shaken until the membranes had dissolved and then radioactivity was measured by scintillation counting. Corrections for varying efficiencies of counting were made with a quench calibration curve.

Chemicals. Unless stated otherwise, diaminopimelic acid was a mixture of about 80% *meso*- and 20% LL-isomers, and was kindly given by Chas. Pfizer & Co., Groton, Connecticut, U.S.A. From it, *meso*- and LL-diaminopimelate were isolated as described by White, Lejeune & Work (1969). DD-Diaminopimelate was isolated from synthetic diaminopimelic acid as described by Saleh & White (1975). [1-¹⁴C]Diaminopimelate was purchased from Calatomic, 54984 Terminal Annex, Los Angeles, California, U.S.A. 3-Hydroxy-2,6-diaminopimelic acid (racemic isomer B; Stewart, 1961) was a gift from Dr C. Gilvarg (Princeton University, U.S.A.); in this isomer the amino groups are in the *meso*-configuration.

RESULTS

All of the enzymic activity measured after the organisms had been made permeable to external diaminopimelate by detergent treatment (White *et al.*, 1964) was easily and reproducibly obtained in a soluble extract prepared with a French pressure cell (Milner, Lawrence & French, 1950) and a motor-driven hydraulic press (American Instruments, Silver Spring, Maryland, U.S.A.).

Effects of lysine, diaminopimelate and pyridoxine on diaminopimelate decarboxylase activities

Lysine. Diaminopimelate decarboxylase activity in exponential-phase organisms decreased when L-lysine was added to the medium (Fig. 1). The maximum apparent repression (about 60%) was at 40 mg l⁻¹. Although higher concentrations of lysine had no greater effect (White *et al.*, 1964), the internal concentration of lysine had not presumably reached its highest possible level because lysine decarboxylase was only found in organisms when lysine in the medium was at 100 mg l⁻¹ or higher (White *et al.*, 1964). D-Lysine (up to 100 mg l⁻¹) caused no repression of diaminopimelate decarboxylase; with D-lysine at 40 mg l⁻¹, enzymic activity was about 25% higher than in minimal medium. Stimulation by D-lysine has been noticed previously (White *et al.*, 1964), but has not been thoroughly studied.

Decarboxylation of 4 mM-*meso*-diaminopimelate was inhibited by 34% when 40 mM L-lysine was added to the assay system with an extract of organisms from minimal medium.

Fig. 1

Fig. 2

Fig. 1. Activity of diaminopimelate decarboxylase in extracts of *E. coli* w grown with various concentrations of diaminopimelic acid in: ●, minimal medium; ■, medium plus pyridoxine (10 mg l⁻¹); ○, medium plus L-lysine (40 mg l⁻¹); ▽, medium plus lysine and pyridoxine. Organisms were grown in shaken flasks and harvested in the mid-exponential phase of growth when the colorimeter reading was about 1.0. Diaminopimelate decarboxylase activities are expressed as a percentage of the activity in minimal medium.

Fig. 2. Activity of diaminopimelate decarboxylase (○) and lysine decarboxylase (▽) in extracts of *E. coli* w grown in minimal medium plus pyridoxine (10 µg ml⁻¹) and various concentrations of lysine. Organisms were grown as described in Fig. 1. Lysine decarboxylase was measured at pH 6.0; its activity was lower at pH 6.8.

Added 40 mM-lysine inhibited diaminopimelate decarboxylase by 38% when the extract was from organisms grown with L-lysine (40 mg l⁻¹).

Lysine plus diaminopimelate. Adding diaminopimelate to the minimal medium had only a slight effect on the diaminopimelate decarboxylase activity (Fig. 1). However, when lysine (40 mg l⁻¹) was in the medium, adding diaminopimelate from 0 to 40 mg l⁻¹ progressively restored enzymic activity almost to the level found in organisms grown without lysine. This increase of activity was not due to the formation of lysine decarboxylase, which would, if it were present as well as diaminopimelate decarboxylase, increase the measured rate of CO₂ evolution from diaminopimelate. No lysine decarboxylase was found at pH 6.0 (optimal for this enzyme) or at pH 6.8 (at which diaminopimelate decarboxylase activity is optimal and is assayed) in extracts of organisms grown with lysine (40 mg l⁻¹) plus diaminopimelate (40 mg l⁻¹).

Either pure *meso*- or pure LL-diaminopimelate gave results similar to those described above for the mixed isomers. These two isomers are interconvertible by the enzyme diaminopimelate epimerase, which is present in *E. coli* (Antia, Hoare & Work, 1957). DD-Diaminopimelate is neither a substrate nor an inhibitor of the epimerase or decarboxylase. Growth of *E. coli* w in shaken flasks with DD-diaminopimelate completely prevented repression of diaminopimelate decarboxylase by lysine, but the enzyme was not induced by the DD-isomer (Table 1).

3-Hydroxy-2,6-diaminopimelate also was neither a substrate nor an inhibitor of diaminopimelate decarboxylase. This compound completely prevented repression of the

Table 1. Activity of diaminopimelate decarboxylase in extracts of *E. coli* strains W, 173-25 and D3 after growth with various additions to the medium

Organisms were grown in minimal medium with various additions, in shaken flasks or in an aerated fermenter (see Methods). Flask cultures were harvested when the colorimeter reading (turbidity) was about 1.0 and extracts were made from the washed organisms. Fermenter cultures were sampled at intervals and the enzymic activities were determined from differential plots (see Fig. 3). Results are for *E. coli* W, except where indicated. Unless stated otherwise, diaminopimelate was a mixture of *meso*- and LL-isomers, and other amino acids were L-isomers. The additions were used throughout at the concentrations given at first mention in the Table.

Addition to growth medium	Diaminopimelate decarboxylase activities [(nmol CO ₂) min ⁻¹ (mg protein) ⁻¹]			
	Flask cultures		Fermenter cultures	
	No pyridoxine in medium	Pyridoxine (10 mg l ⁻¹) in medium	No pyridoxine in medium	Pyridoxine (10 mg l ⁻¹) in medium
None	80 to 100	59	114	50
Lysine (40 mg l ⁻¹)	32	14	25	13
D-Lysine (40 mg l ⁻¹)	105	ND	ND	ND
Diaminopimelate (40 mg l ⁻¹)	90	58	141	79
			12*	
			29†	
<i>meso</i> -Diaminopimelate (40 mg l ⁻¹)	86	ND	ND	ND
LL-Diaminopimelate (40 mg l ⁻¹)	85	ND	ND	ND
DD-Diaminopimelate (40 mg l ⁻¹)	102	ND	ND	ND
3-Hydroxydiaminopimelate (racemic isomer B; 44 mg l ⁻¹)	119	ND	ND	ND
Lysine plus diaminopimelate	81	32	98	32
Lysine plus DD-diaminopimelate	104	ND	ND	ND
Lysine plus 3-hydroxydiaminopimelate	86	ND	ND	ND
Lysine plus threonine (40 mg l ⁻¹)	36	ND	50	ND
DL-Phenylalanine (50 mg l ⁻¹)	90	ND	114	ND
Lysine plus phenylalanine	31	13	ND	ND

ND, Not determined.

* Mutant 173-25.

† Mutant D3.

decarboxylase by lysine, and also caused a slight induction of the enzyme when added to minimal medium in shaken flasks (Table 1). Insufficient DD- or 3-hydroxydiaminopimelate was available to allow experiments with these compounds in fermenter-grown cultures.

Pyridoxine. Previous studies (White *et al.*, 1964) showed that adding pyridoxine to minimal medium decreased diaminopimelate decarboxylase activity in detergent-treated *E. coli* W, and this effect was distinct from the effect of lysine on the enzyme. Activities of diaminopimelate decarboxylase in soluble extracts are also decreased by pyridoxine in the growth medium. Enzymic activity of organisms grown in shaken flasks with pyridoxine was not increased by adding diaminopimelate to the medium, though the activity of the enzyme in organisms grown with pyridoxine plus lysine was raised by adding diaminopimelate (Fig. 1). When pyridoxine was in the growth medium, the maximum repression of diaminopimelate decarboxylase was reached with lysine at 50 mg l⁻¹. Lysine decarboxylase began to be induced only when lysine was at 80 mg l⁻¹ (Fig. 2). Thus the effect of pyridoxine is probably not a consequence of raising the concentration of lysine in the bacteria.

All the experiments so far described were done with exponential-phase cultures grown in shaken flasks; in these, diaminopimelate decarboxylase activity varies with the stage of

Fig. 3

Fig. 4

Fig. 3. Differential plots of diaminopimelate decarboxylase activities in fermenter-grown cultures of *E. coli* w in: ○, minimal medium; ●, medium plus diaminopimelate (44 mg l^{-1}); ▽, medium plus L-lysine (40 mg l^{-1}); ■, medium plus lysine and diaminopimelate. Samples (1 to 2 l culture) were taken, turbidities were measured and extracts were prepared from the washed organisms.

Fig. 4. Uptake of [$1\text{-}^{14}\text{C}$]diaminopimelic acid by suspensions of *E. coli*: ○, strain w; ▽, strain 173-25; ●, strain D3. Procedure was as described in Methods.

growth (White *et al.*, 1964). When organisms were grown in an 11 l fermenter, the rates of enzyme synthesis with various additions to the medium could be compared by 'differential plots' (Monod, Pappenheimer & Cohen-Bazire, 1952) of the amount of enzyme in the culture $[(\text{mu.}/\text{mg protein}) \times \text{turbidity}]$ against the weight of bacteria in the culture (turbidity) at the time of sampling (Fig. 3).

The fermenter cultures showed some differences in diaminopimelate decarboxylase activities from those grown as flask cultures in media of the same composition. In the fermenter, enzyme was formed at a constant differential rate throughout growth; enzymic activity in minimal medium was higher; the depressing effects of lysine and pyridoxine on enzyme synthesis were more marked; diaminopimelate more effectively prevented these effects of both lysine and pyridoxine; and adding diaminopimelate to the minimal medium stimulated enzymic activity (Table 1).

Activity of diaminopimelate decarboxylase in the diaminopimelate-requiring mutants 173-25 and D3, and the uptake of diaminopimelate by these mutants and the wild type

Leive & Davis (1965) isolated from strain 173-25 a second-stage mutant (called D2) that grew quickly in minimal medium supplemented with diaminopimelate alone, and they showed that D2 organisms seemed to differ from 173-25 only by this growth response and by taking up [^{14}C]diaminopimelate more rapidly.

The uptake of [^{14}C]diaminopimelate by washed suspensions of strains w, 173-25 and D3 were compared (Fig. 4). The D3 organisms took up diaminopimelate faster and to a greater extent than did strains w or 173-25 under the conditions of Leive & Davis (1965). The

Fig. 5

Fig. 6

Fig. 5. Disappearance of lysine and diaminopimelate during growth of *E. coli* w. Organisms were grown in shaken flasks at 37 °C with L-lysine hydrochloride (50 mg l⁻¹) or with lysine plus diaminopimelate (50 mg l⁻¹) in minimal medium. Samples were taken, their turbidities were measured, and, after centrifuging, the supernatant liquids were assayed for lysine and diaminopimelate. ○, Growth in minimal medium plus lysine; ●, growth in minimal medium plus lysine and diaminopimelate; ▽, lysine concentration (no diaminopimelate added); ▼, lysine concentration (diaminopimelate added); ■, diaminopimelate concentration. (A separate experiment, not illustrated, showed that the rates of disappearance of diaminopimelate and of growth were unaltered when only diaminopimelate was added to the minimal medium.)

Fig. 6. Persistence of lysine in culture filtrates of *E. coli* w after removing the bacteria. Organisms were grown with lysine, as described in Fig. 5. At intervals, samples were taken, and the organisms were removed by filtering. These sterilized solutions were incubated at 37 °C and assayed at intervals for lysine. ○, Lysine concentration at time of sampling; Δ, □ and ●, lysine concentration in culture filtrates on continued incubation after removal of bacteria; ■, growth. [Similar results, not illustrated, were obtained when diaminopimelate (50 mg l⁻¹) was present in the medium with lysine.]

conditions in a fermenter-grown culture were considerably different, and so it is possible that the rates of uptake of diaminopimelate by organisms in the fermenter were unlike the measured rates.

Both mutants (D3 and 173-25) grew in the aerated fermenter in medium supplemented only with diaminopimelate (44 mg l⁻¹) though 173-25 grew with a longer doubling time (110 min) than D3 or the wild type (40 min). The differential rate of formation of diaminopimelate decarboxylase was 2.5 times higher in D3 than in 173-25, although D3 itself only formed the enzyme at one quarter of the rate of the parent strain (Table 1). Nevertheless, D3 does contain more than sufficient enzyme per mg protein to form lysine quickly enough to double its protein content (assuming one-twentieth of the protein is lysine) in 20 min, which is a shorter period than the observed mean generation time. Adding lysine (40 mg l⁻¹) as well as diaminopimelate had no effect on enzyme synthesis by either mutant.

When the rates of disappearance of lysine and diaminopimelate from the medium of growing cultures of *E. coli* w were compared (Fig. 5), disappearance of each amino acid was unaffected by the other. The disappearance of lysine must represent uptake, because no exoenzyme that degraded lysine was found in culture filtrates (Fig. 6).

Table 2. *Activity of aspartokinase in extracts of E. coli w after growth with various additions to the medium*

Organisms were grown in shaken flasks of minimal medium with various additions. Cultures were harvested in the mid-exponential phase of growth (colorimeter reading about 1.0) and extracts were made from the washed organisms. The isomeric compositions and concentrations of the additions (where not specified) were as given in Table 1. Enzyme activities are expressed as a percentage of the activity in minimal medium.

Addition	Enzyme activities (%)	
	Aspartokinase	Diaminopimelate decarboxylase
None	100*	100†
Lysine	54‡	35
Diaminopimelate	100	100
Lysine plus diaminopimelate	58§	90
Pyridoxine	100	65
Threonine	92	ND
Lysine plus threonine	38	40
Phenylalanine	100	100
L-Tryptophan (40 mg l ⁻¹)	100	100
Lysine plus phenylalanine	39	35
Pyridoxine plus diaminopimelate	100	65

ND, Not determined.

* About 80 mu. (mg protein)⁻¹.

† About 90 mu. (mg protein)⁻¹.

‡ 50 % when 1 mM-lysine added to assay system.

§ 51 % when 1 mM-lysine added to assay system.

Activity of aspartokinase in E. coli w

Lysine and other amino acids have differing complex regulatory effects on aspartokinase activity in various strains of *E. coli* (see review by Umbarger, 1969). Patte, Loviny & Cohen (1965) reported that phenylalanine enhanced the inhibitory effect of lysine on the lysine-sensitive aspartokinase of *E. coli*, and this might explain the slight repression of diaminopimelate decarboxylase by phenylalanine found by White *et al.* (1964). Decrease in the activity of aspartokinase might possibly lower the internal concentration of diaminopimelate, which might in turn then induce less decarboxylase. However, unless aspartokinase is totally inhibited *in vivo*, decreased activity of this enzyme will not necessarily lead to a decreased flow of carbon to diaminopimelate. Lysine also represses aspartic semialdehyde dehydrogenase (Cohen & Patte, 1963) and inhibits the condensation reaction between aspartic semialdehyde and pyruvate (Yugari & Gilvarg, 1962).

The activities of aspartokinase and diaminopimelate decarboxylase were compared in extracts of *E. coli w* that had been grown with various additions to the medium (Table 2). Phenylalanine added alone to the medium did not affect either enzyme, even when organisms were used from freeze-dried material that had been preserved at the time (1962) when the repression by phenylalanine was first noticed; tryptophan was also ineffective. Phenylalanine slightly increased the repression of aspartokinase by lysine and partly inhibited this enzyme as well (Table 3). Threonine alone was not inhibitory, but threonine plus lysine caused almost total inhibition of aspartokinase *in vitro*. Nevertheless, when these two amino acids were added to the minimal medium, they caused little more repression of aspartokinase than did lysine alone, and did not prevent formation of diaminopimelate decarboxylase. The flow of carbon to diaminopimelate may therefore be chiefly controlled at the condensation of aspartic semialdehyde with pyruvate, and at aspartic semialdehyde dehydrogenase.

Table 3. Activity of aspartokinase in an extract of *E. coli* w with various additions to the assay system

Additions to the assay system were made with an extract of organisms grown in minimal medium [uninhibited aspartokinase activity 80 mu. (mg protein)⁻¹]. The isomers of the amino acids (where not specified) were as given in Tables 1 and 2. Aspartokinase activity is expressed as a percentage of the activity measured when no additions were made to the assay system.

Addition	Aspartokinase (%)
None	100
Lysine (1 mM)	54*
Diaminopimelate (10 mM)	100
Lysine plus diaminopimelate	60
Threonine (10 mM)	100
Lysine plus threonine	15
Phenylalanine (10 mM)	62
Tryptophan (10 mM)	100
L-Tyrosine (10 mM)	59
Lysine plus phenylalanine	39

* No greater inhibition with concentrations of lysine up to 10 mM.

Lysine partly repressed aspartokinase in *E. coli* w and partly inhibited this enzyme in extracts of organisms grown in minimal medium. The residual aspartokinase activity of organisms grown with lysine was not inhibited by lysine. Thus, the lysine-sensitive enzyme is the one that is repressed by lysine. Patte, Boy & Borne (1974) suggested that in *E. coli* K12 the synthesis of diaminopimelate decarboxylase was regulated by the activity of the lysine-sensitive aspartokinase. This is clearly not the case in the present study: lysine plus diaminopimelate almost completely repressed the lysine-sensitive aspartokinase, but had very little effect on diaminopimelate decarboxylase; pyridoxine repressed the decarboxylase, but had no effect on aspartokinase. The experiments of Patte *et al.* (1974) are consistent with the view that a lowered aspartokinase activity may decrease the internal concentration of diaminopimelate and hence diminish synthesis of diaminopimelate decarboxylase. However, a difficulty in making comparisons is that Patte *et al.* (1974) used *E. coli* K12 and found very much lower diaminopimelate decarboxylase activities [only 5 mu. (mg protein)⁻¹ in unrepressed bacteria] than are present in *E. coli* w.

DISCUSSION

Growth of *E. coli* w in the presence of lysine decreases the activity of diaminopimelate decarboxylase found in extracts, but this effect of lysine is almost completely overcome if diaminopimelate is also present in the medium. None of the other common natural amino acids abolished lysine repression of this enzyme (White *et al.*, 1964). Diaminopimelate does not stop the uptake of lysine (Fig. 5) and must, therefore, be able to overcome the apparent lysine repression at some internal site(s). Three interpretations seem possible:

(i) Lysine regulates diaminopimelate decarboxylase activity by inhibition (White & Kelly, 1965; Rosner, 1975) rather than by repression of enzyme synthesis, and diaminopimelate might overcome or prevent the inhibition. This hypothesis is improbable because the dilution of the bacterial cytoplasm during extraction of the enzyme should decrease the concentration of lysine to a subinhibitory level (unless the lysine were very firmly bound to the diaminopimelate decarboxylase). Furthermore, the repressed decarboxylase from lysine-grown

organisms was just as sensitive to inhibition by lysine as was the enzyme from organisms grown in minimal medium.

(ii) Lysine decreases the rate of synthesis of diaminopimelate decarboxylase but diaminopimelic acid prevents this repression.

(iii) Diaminopimelate induces the formation of diaminopimelate decarboxylase. Lysine might cause an apparent repression by decreasing the internal concentration of diaminopimelate by inhibition and/or repression of enzymes in the pathway of diaminopimelate biosynthesis.

A firm choice cannot yet be made between these hypotheses. The third most easily accounts for the experimental findings reported in this paper and does not demand any novel competitive interaction with lysine such as the first or second hypothesis requires.

The regulatory effects of lysine must not stop diaminopimelate biosynthesis completely, otherwise lysine in the medium would prevent formation of peptidoglycan. In contrast, there is no obvious reason why lysine should not repress diaminopimelate decarboxylase completely (which it does not), if it had a direct controlling effect on synthesis of this enzyme.

Growth of several strains of *E. coli* and other bacteria in flasks of minimal media plus diaminopimelate did not increase formation of diaminopimelate decarboxylase (White *et al.*, 1964). However, diaminopimelate can accelerate enzyme formation when *E. coli* grows in an aerated fermenter. Diaminopimelate also behaves like an inducer when it is added in gradually increasing concentrations to medium containing lysine. Probably the very slow rate of uptake of diaminopimelate by *E. coli* (Leive & Davis, 1965) prevents the induction being more striking.

The diaminopimelate-requiring mutant 173-25 grows only slowly in minimal medium supplemented with diaminopimelate, but grows as quickly as its parent strain (w) with lysine plus diaminopimelate. Only about one-twentieth as much diaminopimelate needs to be taken up when it is wanted solely for peptidoglycan synthesis and is not required as a precursor of lysine. Consequently, the low rate of entry of diaminopimelate limits the rate of growth of 173-25 only when lysine is not provided. The very low activity of diaminopimelate decarboxylase in 173-25 grown with only a diaminopimelate supplement is consistent with a low internal concentration of the presumed inducer. The lack of any repression by lysine (plus diaminopimelate) with 173-25 is also consistent with the effect of lysine in the wild type being due to partial inhibition of diaminopimelate biosynthesis. However, even in the wild type, the effect of lysine in the medium is only slight when diaminopimelate is present. Mutant D3 transports diaminopimelate quickly enough to grow with diaminopimelate (in the absence of lysine) as rapidly as the wild type. The activity of diaminopimelate decarboxylase in the mutant D3 was almost three times higher than in 173-25 though it was still only one-quarter of that of the wild type.

A possible explanation can now be advanced for the repression of diaminopimelate decarboxylase that is caused by pyridoxine. White & Kelly (1965) showed *in vitro*, and Bukhari & Taylor (1971) showed *in vivo*, that in *E. coli* the attachment between the diaminopimelate decarboxylase apoenzyme and its cofactor, pyridoxal phosphate, is very weak. When organisms grow with pyridoxine, the internal concentration of pyridoxal phosphate might become sufficient to activate every molecule of diaminopimelate decarboxylase protein that is synthesized. The catalytic activity of the holoenzyme may regulate its own further production, by decreasing the internal concentration of diaminopimelate or, less probably, by increasing the internal concentration of lysine. When pyridoxal phosphate is not in excess, the organisms may produce a surplus of apoenzyme (which is measured

during enzyme assay) before enough active holoenzyme accumulates and shuts off further synthesis of apoenzyme. In other words, the enzymic activity measured after growth with pyridoxine may be a better indication of the true activity of the intracellular enzyme.

Kelleher & Heggeness (1976) showed, with mutants of *E. coli* K12, that repression of diaminopimelate decarboxylase apparently by lysine was less effective when the organisms contained additional copies of the *lysA* gene, which codes for diaminopimelate decarboxylase. The authors did not consider the possibility of induction by diaminopimelate but suggested that synthesis of this enzyme is regulated by lysine in combination with a small number of lysine-repressor molecules that are insufficient to repress additional copies of the structural gene. The findings of Kelleher & Heggeness (1976) are in fact equally consistent with the hypothesis that diaminopimelate regulates synthesis of the enzyme by combining with active repressor molecules that are formed in limited numbers.

The results described in this paper can more readily be explained with the hypothesis that diaminopimelate decarboxylase is induced by its substrate, rather than directly repressed by its product. In general, biosynthetic enzymes are not controlled by induction (Sanwall, 1970), though all other bacterial amino-acid decarboxylases (which are catabolic) are inducible enzymes (Gale, 1946). Possibly diaminopimelate decarboxylase was originally evolved as the first step in diaminopimelate degradation by organisms that had already gained the ability to make diaminopimelate for wall synthesis but previously had required lysine for growth (see review by Vogel, Thompson & Shockman, 1970). The control mechanism of a catabolic enzyme might have been retained even though the prime function has become biosynthetic.

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