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Aspartokinase of a lysine producing mutant of *Micrococcus glutamicus*

MEENA LAKSHMAN, B. C. SHENOY and M. R. RAGHAVENDRA RAO
Discipline of Biochemistry and Applied Nutrition, Central Food Technological Research Institute, Mysore 570 013

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Abstract. Aspartokinase from *Micrococcus glutamicus* AEC RN-13-6/1 [a homoserine requiring, S-(2–aminoethyl)-L-cysteine resistant, lysine producing strain] was purified 71 fold. The partially purified enzyme was inhibited by L-lysine. L-threonine, L-methionine, L-isoleucine, L-valine and L-phenylalanine activated the enzyme and reversed the inhibition by L-lysine. Aspartokinase activity was not derepressed by growth–limiting concentrations of L-threonine and/or L–methionine. It was not repressed by an excess of L-lysine (20 mM) and/or L-isoleucine (15.3 mM). The degree of activation or inhibition by amino acids was dependant on the composition of the growth medium. This observation is in contrast with the enzyme from the original (non–lysine–producing) strain which was inhibited by lysine or threonine and in a concerted manner by threonine plus lysine.

Keywords. Aspartokinase; *Micrococcus glutamicus.*

Introduction

The properties of aspartokinases (adenosine triphosphate (ATP): L-aspartate-4-phosphotransferase, EC 2.7.2.4) of different strains of *Micrococcus glutamicus* have been investigated only in crude extracts of cells (Kase and Nakayama, 1974; Nakayama *et al.,* 1966; Zaitseva and Chernysheva, 1975). Amino acid auxotrophs of *M. glutamicus* were found to have aspartokinase activities sensitive to concerted/synergistic feedback inhibition by lysine plus threonine, although the response of the enzyme to lysine or threonine alone differed in these various strains (Kase and Nakayama, 1974; Nakayama *et al.,* 1966; Zaitseva and Chernysheva, 1975). Screening for resistance to S-(2-aminoethyl)-L-cysteine (AEC), a lysine analogue, in an organism with aspartokinase activity sensitive to concerted/synergistic feedback inhibition by lysine plus threonine yielded mutants with a variety of feedback inhibition patterns (Hermann *et al.,* 1972; Kase and Nakayama, 1974; Shiio *et al.,* 1970; Tosaka *et al.,* 1978). Repression of the aspartokinase activity of *M. glutamicus* has not been previously investigated.

Abbreviations used: AEC or aminoethyl cysteine, S-(2-aminoethyl)-L-cysteine; Lys, L-lysine; L-Asp, L-aspartate; Thr, L-threonine; IIe,.L-isoleucine.

The partial purification, properties and repression of aspartokinase from *M. glutamicus* AEC RN-13-6/1 (homoserine^{-} , AEC^{R}; a lysine excreting strain) are reported in this paper. The feedback inhibition properties of the aspartokinase of this organism differ from those of the AEC^{R} mutants described earlier (Kase and Nakayama, 1974).

Materials and methods

Chemicals

DL-aspartic-β-semialdehyde was prepared by ozonolysis of DL-C-allylglycine (Black and Wright, 1955). Alumina type 305, DEAE-Sephadex, ATP and all amino acids were purchased from Sigma Chemical Company, St. Louis, Missouri, USA. L-threonine used in growth media was a product of Kyowa Hakko Kogyo Limited, Tokyo, Japan. All other chemicals were of the highest purity available.

Bacteria and growth

M. glutamicus AEC RN-13-6/1 was a gift from Dr V. C. Vora, Central Drug Research Institute, Lucknow. It was obtained from *M. glutamicus* No. 267 (homoserine^{-}) by ultraviolet (UV) mutagenesis and selection on plates with medium B (Sano and Shiio, 1967) containing aminoethyl cysteine (2 mg/ml) and L-threonine (2 mg/ml). *M. glutamicus* No. 267 was itself derived from *M. glutamicus* ATCC 13059 by UV mutagenesis (V. C. Vora, personal communication). *M. glutamicus* AEC RN-13-6/1 excreted 16 g/1 lysine HCl under favourable growth conditions. Under identical growth conditions, the lysine HCl content of the culture filtrate of *M. glutamicus* ATCC 13059 did not rise above the 60 mg/ml level observed in uninoculated media (Lakshman, 1980).

M. glutamicus AEC RN-13-6/1 was maintained on slants of medium B (Sano and Shiio, 1967) containing aminoethyl cysteine (2 mg/ml), L-threonine (2 mg/ml) and DL-methionine (50 µg/ml). Medium B contained ammonium sulphate, 1.5 g; urea, 1.5 g; KH_2PO_4, 1 g; K_2HPO_4, 3 g; $MgSO_4.7H_2O$, 0.1 g; $CaCl_2.2H_2O$, 0.001 g; *d*-(+)-biotin,30µg; thiamine- HCl, 100 µg; trace element solution, 1 ml and distilled water to a final volume of 1000 ml. The pH was adjusted to 7.0 before sterilization. Glucose was sterilized separately as a 25% solution and added to the medium to 0.5%. The trace element solution contained $ZnSO_4.7H_2O$, 8.8 g; $FeCl_3.6H_2O$, 0.97 g; $CuSO_4.5H_2O$, 0.393 g; $Na_2B_4O_7.10H_2O$, 88 mg and distilled water, 1000 ml. Cells were grown with shaking (270 rpm) at 30°C, in one-litre Erlenmeyer flasks containing 200 ml medium B suplemented with L-threonine (2 mg/ml) and DL-methionine (50 µg/ml). They were harvested in the late exponential phase by centrifugation (10,000 g, 20 min), washed twice with 0.9% NaCl at 4°C and stored frozen as a pellet.

For repression experiments, cells in the exponential phase of growth in medium B containing L-threonine (2 mg/ml) and DL-methionine (50 µg/ml), were washed once with sterile medium B (glucose omitted), suspended in sterile medium B to the original density and inoculated into medium B with various amino acid suplements.

The cells were harvested towards the end of the exponential phase of growth and processed as above.

Medium C (V. C. Vora, personal communication), a growth medium favourable for lysine production, contained glucose, 15 g; NH_4C1, 1 g; $MgSO_4.7H_2O$, 0.02g; corn steep liquor, 5 g; urea, 0.25 g; K_2HPO_4, 0.10 g and distilled water, 1000 ml. Corn steep liquor, urea and K_2HPO_4 were autoclaved separately. Corn steep liquor was clarified before use as described elsewhere (Lakshman, 1980). The various components were mixed and the pH adjusted to 7.0 just before inoculation.

Enzyme assays

Aspartokinase was assayed by estimating the aspartate-β-hydroxamate formed from β-aspartyl phosphate (Black, 1962). The reaction mixture contained in 0.5 ml, aspartate-Tris (pH 7.5), 50 µmol; ATP (pH 7.5), 10 µmol; $MgCl_2$, 10 µmol; $NH_2OH.KC1$ (pH 7.5) 320 µmol and enzyme. The reaction was allowed to proceed for 40 min at 37°C, and terminated by the addition of 0.75 ml of ferric chloride reagent (10% ferric chloride.$6H_2O$ and 3.3% trichloroacetic acid in 0.7 N HCl). The precipitated protein were removed by centrifugation and the absorbance of the supernatant solution was measured at 540 nm in a Beckman spectrophotometer model DU-2. A unit of enzyme activity catalyzes the formation of 1 µmol of aspartate-β-hydroxamate per min at 37°C.

Dihydrodipicolinate synthetase [L-aspartate-β-semialdehyde hydrolyase (adding pyruvate and cyclizing) EC 4.2.1.52] was assayed by measuring the change in absorbance at 270 nm in a Beckman spectrophotometer model DU–2, in the presence of imidazole (Yugari and Gilvarg, 1965). The assay system contained per ml, imidazole–HCl buffer (pH 7.4), 100 µmol; sodium pyruvate, 15 µmol; DL-aspartic-β-semialdehyde, 1 µmol and the enzyme. A unit of enzyme activity has been defined as the amount of enzyme producing an increase in absorbance of 1.00 per min at 270 nm (10 min after addition of all components). Diaminopimelate decarboxylase (*meso*-2, 6-diaminopimelate carboxylase, EC 4.1.1.20) was assayed by measuring the rate of diaminopimelate disappearance (White, 1971). The assay system contained per ml, Tris-HCl buffer (pH 7.5), 100 µmol, diaminopimelate, 5 µmol, pyridoxalphosphate 50 nmol and enzyme. The reaction was started by the addition of the substrate and incubation was for 20 min at 37°C. At zero time and after 20 min, 0.1 ml of the reaction mixture was withdrawn into tubes containing 0.4 ml water plus 0.4 ml glacial acetic acid. 0.5 ml of the ninhydrin reagent (250 mg ninhydrin in 6 ml glacial acetic acid and 4 ml 0.6 M phosphoric acid) was added. The colour was developed at 37°C for 90 min and measured at 440 nm as for aspartokinase. A unit of enzyme activity catalyzes the disappearance of 1 µmol of diaminopimelate per min at 37°C.

Analytical methods

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

Results

Purification of aspartokinase

All the purification steps were carried out at 4-8°C. Frozen *M. glutamicus* cells were thawed, ground with three times their weight of alumina and extracted with 0.1 M potassium phosphate buffer (pH 7.0) containing 20 mM 2-mercaptoethanol, 1 mM L-lysine and 1 mM L-threonine (phosphate-mercaptoethanol-Lys-Thr buffer 5 ml/g wet-packed cells). The resulting suspension was centrifuged at 10,000 *g* in a Sorvall RC-2B centrifuge for 10 min and the clear supernatant termed as crude extract. The crude extract was centrifuged at 103,000 *g* for 60 min in a Beckman Model L2-65B ultracentrifuge. A 25% (w/v) solution of streptomycin sulphate in posphate-mercaptoethanol-Lys-Thr buffer was added slowly to the clear 103,000 *g* supernatant to give a final streptomycin sulphate concentration of 3%. Material which precipitated on standing overnight was removed by centrifugation at 27,000 *g* for 20 min. The supernatant was fractionated with solid ammonium sulphate at pH 7.0. After keeping for 60 min, the precipitated proteins were collected by centrifugation at 27,000 *g* for 20 min. Proteins precipitating between 50 and 60% saturation with ammonium sulphate were taken up in phosphate-mercaptoethanol-Lys-Thr buffer and dialyzed against the same buffer for 3 h with rapid stirring and change of buffer every 30 min. The dialyzed enzyme was loaded

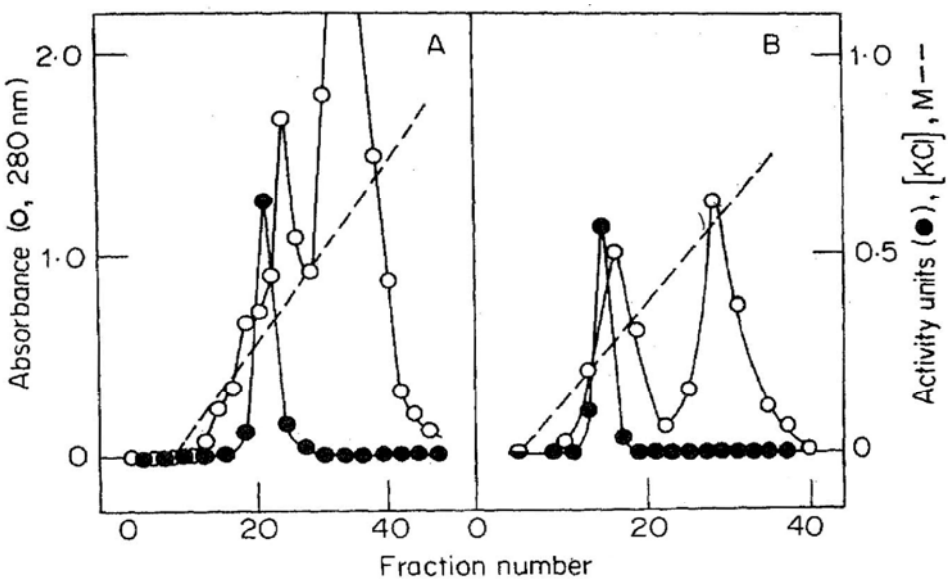

Figure 1. DEAE-Sephadex chromatography of aspartokinase,
A. Fraction 3 (8 ml) (table 1) was loaded on a column (1×23 cm) of DEAE-Sephadex A-50 equilibrated with phosphate-mercaptoethanol-Lys-Thr buffer. The two chambers, of the gradient apparatus contained 75 ml of the above buffer and buffer containing 1 M KCl, respectively. Fractions (3.2 ml) were collected at 5 min intervals.

B. Fraction 4 (20 ml) (table 1) was chromatography under the conditions described above except that phosphate -mercaptoethanol -isoleucine buffer was used instead of the buffer containing Lys and Thr. Fractions (2.2 ml) were collected at 5 min intervals.

on a column (1×23 cm) of DEAE-Sephadex equilibrated with phosphate mercaptoethanol-Lys-Thr buffer. The column was washed with the same buffer until the absorbance at 280 nm was negligible. A linear gradient of KCl (0 to 1 M) in the same buffer was then applied. Fractions containing aspartokinase activity were pooled, dialyzed as described above against 0.1 M potassium phosphate buffer (pH 7.0) containing 20 mM 2-mercaptoethanol and 1 mM L-isoleucine and rechromatographed on DEAE-Sephadex (figure 1). A summary of the purification procedure is given in table 1. The resultant enzyme preparation was not homogeneous as evidenced by the electrophoretic pattern on 7.5% Polyacrylamide gels at pH 8.3. Further purification was not possible, although other procedures were tried. Chromatography on Sephadex G-200 and Sepharose-4B (and its derivatives) caused inactivation of the enzyme.

Table 1. Partial purification of aspartokinase from *M. glutamicus* AEC RN–13–6/1

Fraction	Total protein (mg)	Total activity (units)	Specific activity m units/mg protein)
10,000 *g* supernatant	638 [a]	10.9	17
103,000 *g* supernatant	434	11.4	26
50-60% saturation ammonium sulphate precipitate	91	5.2	57
DEAE-Sephadex I Chromatography	7	4.3	640
DEAE-Sephadex II Chromatography	3.1	3.8	1212

[a] From 18 *g* wet-packed cells

Properties of aspartokinase

Aspartokinase activity was stabilized by inclusion of L-lysine, L-lysine plus L-threonine, L-isoleucine or L-aspartate in the buffers used for extraction or storage. The enzyme activity was completley lost within five days at 4°C in 0.1 M potassium phosphate buffer (pH 7.0) containing 20 mM 2-mercaptoethanol. The enzyme lost 50% activity in three weeks in phosphate-mercaptoethanol-Lys-Thr buffer or phosphate-mercaptoethanol-Ile buffer at 4°C; 50% activity was lost in two weeks in 0.1 M potassium phosphate buffer (pH 7.0) containing 20 mM 2-mercaptoethanol and 5 mM L-aspartate at 4°C. The crude enzyme was optimally active between pH 7.35 and 7.5. Mg^{2+}ions were required for activity. KCl was not required for activity and added KCl upto 640 mM had no effect. The enzyme was markedly stimulated by ammonium sulphate (150 mM), but K_2SO_4, Na_2SO_4, NH_4C1 and NaCl at the same concentration had no effect.

The aspartokinase activity of *M. glutamicus* AEC RN-13-6/1 in partially purified preparations was inhibited by L-lysine. L-threonine, L-methionine and L-isoleucine stimulated the activity and reversed the L-lysine inhibition (table 2). In addition, L-valine or L-phenylalanine also stimulated the activity and reversed the inhibition by L-lysine. L-valine was the most effective amino acid in reversing the inhibition by L-lysine (data not shown). The effects of L-lysine, L-threonine and L-lysine plus L-threonine were the same in the presence and absence of ammonium sulphate (150 mM).

Table 2. Effect of amino acids on the aspartokinase activity of *M.glutamicus* AEC RN–13–6/1.

Additions (10 mM)	Per cent activity			
	Partially purified enzyme [a]	Crude enzyme [b]		
		Growth medium 2	Growth medium 7	Growth medium C
None	100	100	100	100
L-Lys	27	39	29	40
L-Thr	150	313	242	137
L-Met	173	344	240	167
L-Ile	183	352	266	179
L-Lys + L-Thr	59	124	92	69
L-Lys + L-Met	72	157	109	68
L-Lys + L-Ile	100	216	157	95
L-Lys + L-Thr + L-Met + L-Ile	96	ND	ND	96
L-Thr + L-Met	ND	344	220	166

[a] Fraction 4. table 1 was used as the enzyme.

[b] Crude extracts of cells were prepared in phosphate–mercaptoethanol–asparate buffer and dialyzed against the same buffer before use.

Composition of growth media 2 and 7 are shown in table 3.

ND—Not determined.

Repression of aspartokinase

Aspartokinase was not derepressed by growing cells with limiting concentrations of L-threonine and/or L-methionine (table 3). Added L-lysine (20 mM) and/or L-isoleucine (15.3 mM) had no significant effect on the levels of the enzyme. The enzyme was significantly repressed in medium C.

Table 3. Effect of growth medium composition on the levels of the aspartokinase activity of *M. glutamicus* AEC RN-13-6/1.

Medium	Supplements to growth medium	Relative specific activity (%)
B	L-Thr (2 mg/ml) + L-Met (2 mg/ml)	100 (18 [a])
	L-Thr (60 µg/ml) + L-Met (10 µg/ml)	71
	L-Thr (2 mg/ml) + L-Met (10 µg/ml)	74
	L-Thr (60 µg/ml) + L-Met (2 mg/ml)	74
	L-Thr (2 mg/ml) + L-Met (2 mg/ml) + L-Lys (3 mg/ml)	106
	L-Thr (2 mg/ml) + L-Met (2 mg/ml) + L-Ile (2 mg/ml)	93
	L-Thr (2 mg/ml) + L-Met (2 mg/ml) + L-Ile (2 mg/ml) + L-Lys (3 mg/ml)	97
C [b]		31

[a] Specific activity in m units per mg protein.

[b] Medium C has been described in Materials and methods. Cells were harvested in the late exponential phase.

The enzyme was assayed in dialyzed crude extracts. Similar values, less marked, were obtained with undialyzed crude extracts. Phosphate-mercaptoethanol-aspartate buffer was used for extraction and dialysis.

Total activity paralleled cell yield: 2.5-4.0g/ 1 in media 2-4; 7.0-9.0g/l in media 1 and 5-7 and 17-22 g/1 in medium C.

Aspartokinase activities of cells grown in media 2, 7 and C responded to feedback modifiers essentially like the partially purified enzyme (table 2). The enzyme from cells grown in media 2 and 7 was activated to greater extents by L-threonine, L-methionine or L-isoleucine; reversal of L-lysine inhibition by these amino acids was correspondingly greater. The response to feedback modifiers was the same in the exponential phase and the mid and late stationary phases of growth.

Dihydrodipicolinate synthetase and diaminopimelate decarboxylase

Dihydrodipicolinate synthetase and diaminopimelate decarboxylase of *M. glutamicus* AEC RN-13-6/1 were not repressed by an excess of L-threonine, L-methionine, L-lysine or L-isoleucine in the growth medium (table 4). Dihydrodipicolinate synthetase was insensitive to the amino acids of the aspartate family, added singly or in combinations at 10 mM, whereas diaminopimelate decarboxylase was inhibited by L-lysine and to a greater extent by a combination of lysine, threonine, methionine and isoleucine (table 5).

Table4. Effect of compositionof growth medium on levels of dihydrodipicolinate synthetase and diaminopimelate decarboxylase of *M. gutamicus* AEC RN–13–6/1.

Composition of media	Relative specific activity (%)	
	Dihydrodipicolinate synthetase	Diaminopimelate decarboxylase
Medium B + L-Thr (2 mg/ml) + L-Met (2 mg/ml)	100 (0.110 [a])	100 (0.018 [a])
Medium B + L-Thr (60 µg/ml) + L-Met (10 µg/ml)	90	71
Medium B + L-thr (2 mg/ml) + L-Met (10 µg/ml)	88	117
Medium B + L-Thr (60 µg/ml)+ L-Met (2 mg/ml)	89	67
Medium B + L-Thr (2 mg/ml) + L-Met (2 mg/ml) + L-Lys (3 mg/ml)	96	116
Medium B + L-Thr (2 mg/ml) + L-Met (2 mg/ml) + L-Ile (2 mg/ml)	100	100
Medium C	64	139

[a] Specific activity in units per mg protein (Dl+*meso*)–diaminopimelate containing 53% of the *meso*- isomer was used for assay.

Crude extracts, dialyzed against 0.1 M potassium phosphate buffer (pH 7.5) containing 20 mM 2–mercaptoethanol, were used for assay.

Total activity paralleled cell yield (table 3).

Table 5. Effect of amino acids on dihydrodipicolinate synthetase and diaminopimelate decarboxylase activities.

Additions to reaction mixture (10 mM each)	Relative specific activity (%)	
	dihydrodipicolinate synthetase	Diaminopimelate decarboxylase
None	100 (0.110 [a])	100 (0.018 [a])
L-Lys	100	43
L-Thr	98	94
L-Met	112	94
L-Ile	98	100
L-Lys + L-Thr + L-Met + L-Ile	98	25

[a] Specific activity in units per mg protein.

Discussion

Selection for resistance to aminoethyl cysteine yields mutants either with derepressed levels of aspartokinase (Hermann *et al.,* 1972), altered sensitivity of aspartokinase to feedback modifiers (Hermann *et al.,* 1972; Jegede *et al.,* 1976; Shiio *et al.,* 1970; Tosaka *et al.,* 1978), altered sensitivity of dihydrodipicolinate synthetase to feedback inhibitors (Hermann *et al.,* 1972) or altered lysine transport systems (Halsall, 1975).

The aspartokinase activity of *M. glutamicus* ATCC 13059, the parent strain of the mutant used here, was inhibited by L-lysine, slightly inhibited or activated by L-threonine depending on the growth medium and subjected to concerted feedback inhibition by L-lysine plus L-threonine (table 6). The enzyme was stimulated by L-isoleucine, L-valine, L-methionine and L-phenylalanine; these amino acids reversed the multivalent inhibition by L-lysine plus L-threonine (unpublished data). The enzyme therefore resembled the aspartokinase of *Brevibacterium flavum* No. 2247 (Shiio and Miyajima, 1969). The aspartokinase activity of the AEC^R mutants

Table 6. Effect of amino acids on the aspartokinase activity in crude extracts of cells of different *M. glutamicus* strains.

Strain	Per cent activity in the presence of (mM)							Effect of L-Ile on multivalent inhibition by L-Lys+L-Thr	Reference
	L-Lys		L-Thr		L-Lys+ L-Thr		L-Ile		
	1	10	1	10	1 each	10 each	10		
ATCC 13059	85	40	96	82	32	19	173	Reverses	Lakshman, 1980
AEC RN-13-6/1	86	40	134	137	118	69	179	Reverses	Lakshman, 1980
901 (homoserine)		94		123		52	123	Nil	Nakayama *et al.*, 1966
KY 9159 (Met^-)	86	77.5	119	176	86.2	22.1			Kase and Nakayama, 1974
KY 10440 (Met^-, AHV^R, AEC^R)	115	117	174	295	128	31.3			Kase and Nakayama, 1974
KY 10251 (Met^-, AHV^R, AEC^R)		111		107.3		95			Kase and Nakayama, 1974

Activity in the absence of added amino acids is taken as 100%.

All assays were performed in the absence of ammonium sulphate. The enzyme from ATCC 13059 was assayed at pH 7.0 AEC RN-13-6/1 at pH 7.5 and others at pH 8.0.

Cells of ATCC 13059 and AEC RN-13-6/1 were grown in medium C.

The (Met^-, AHV^R) parent strain of KY 10440 responded to amino acids like KY 9159, whereas activation by L-threonine was lost in the (Met^-, AHV^R) parent strain of KY 10251.

AHV-L-α-amino-β -hydroxyvaleric acid.

derived from these two organisms, *M. glutamicus* AEC RN-13-6/1 and *B. flavum* FAI-30, are very similar to one another in their response to lysine, threonine and lysine plus threonine. While the property of stimulation by isoleucine and reversal of lysine inhibition by this amino acid was retained in the *M. glutamicus* mutant, this property was lost in the mutant of *B. flavum* (Shiio *et al.,* 1970). The patterns of desensitization to lysine and threonine inhibition are different in *M. glutamicus* AEC RN-13-6/1 and in the AEC^R mutants of *M. glutamicus* KY 9159 (table 6). This is probably due to the different methods employed in the selection of AEC^R mutants. The aspartokinase activities of various strains of *M. glutamicus* differ slightly in their response to the aspartate family of amino acids (table 6). Considering that the degree of inhibition or activation by feedback modifiers depends on the pH of the assay (Zaitseva and Chernysheva, 1975), the presence or absence of ammonium sulphate in the assay medium (Kase and Nakayama, 1974) and on growth medium composition, the significance of observed differences between strains should be evaluated carefully.

No genetic derepression of aspartokinase activity was detected in *M. glutamicus* AEC RN-13-6/1 (Lakshman, 1980). The specific activity of aspartokinase in crude extracts of *M. glutamicus* KY 10440 was 1.3 times higher than in its parent strain KY 9159 (Kase and Nakayama, 1974). On the other hand, the aspartokinase activity in crude extracts of the AEC^R mutant of *B. flavum* was 2.5 times higher than in the parent AEC-sensitive strain (Shiio *et al.,* 1970).

Isofunctional aspartokinases with differing repression and feedback inhibition patterns can be detected by observing feedback inhibition pattern in cells obtained from different growth media or during purification. They may also be detected by observing feedback inhibition patterns at different growth stages, provided the turnover rates of these enzymes differ. The data presented here, of the purification as well as the similarity in the pattern of feedback inhibition in different growth media and growth stages, failed to reveal more than one aspartokinase in *M. glutamicus* AEC RN-13-6/1. These experiments do not however conclusively rule out the possibility of the existence of multiple aspartokinases in this organism.

Dihydrodipicolinate synthetase in crude extracts of *M. glutamicus* has been reported to be insensitive to L-lysine (Nakayama *et al.,* 1966). Diaminopimelate decarboxylase of *M. glutamicus* was reported to be repressed slightly (10-20%) by 40 mM L-lysine and inhibited by L-lysine, competitively with respect to diaminopimelate (Drazic and Vitale, 1974). Repression of dihydrodipicolinate synthetase and the effect of amino acids other than L-lysine on the level of diaminopimelate decarboxylase have not been investigated. Data presented in this communication support and extend the earlier observation (table 4). Together with the observation that L-lysine, methionine, threonine and isoleucine have no repressive effect on aspartokinase (table 3), these data suggest that feedback inhibition of aspartokinase activity is the most important mechanism in end-product regulation of L-lysine biosynthesis in *M. glutamicus* AEC RN-13-6/1. Furthermore, the levels and regulatory properties of dihydrodipicolinate synthetase and diaminopimelate decarboxylase were the same in *M. glutamicus* AEC RN-13-6/1 and *M. glutamicus*

ATCC 13059 (Lakshman, 1980). Therefore, the major difference in regulation of lysine biosynthesis in these strains, which provides a plausible explanation for lysine over-synthesis by the former, was the feedback inhibition of aspartokinase.

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