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THE ASPARTATE KINASE OF *PSEUDOMONAS PUTIDA* REGULATION OF SYNTHESIS AND ACTIVITY

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SUMMARY

A single aspartate kinase (ATP:L-aspartate 4-phosphotransferase, EC 2.7.2.4) of *Pseudomonas putida* has been obtained in a partially purified state. The enzyme is inhibited allosterically by L-lysine and L-threonine individually and is also subject to a concerted feedback inhibition by these amino acids.

Some of the properties of the enzyme, the kinetic constants and approximate molecular weight, the type of inhibition by L-lysine and L-threonine and their concerted feedback inhibition, the effect of lysine and threonine analogues on enzyme activity and the protection of the enzyme by the inhibitors against thermal inactivation, are described.

Investigation of regulation by feedback inhibition and repression revealed that enzyme synthesis is not only repressed when cells are growing in the presence of the inhibitors but that the enzyme synthesized under these conditions is sensitive neither to lysine or threonine nor to their concerted feedback inhibition.

The regulation and some other particularities of the enzyme are discussed.

INTRODUCTION

β-Aspartate kinase (ATP:L-aspartate 4-phosphotransferase, EC 2.7.2.4) is the first enzyme in the branching biosynthetic pathway leading from aspartate to diaminopimelic acid, lysine, methionine, threonine and isoleucine in bacteria[1]. This enzyme catalyzes the phosphorylation of the β-carboxyl function of aspartic acid by ATP, according to the following equation:

$$\text{Aspartate} + \text{ATP} \overset{Mg^{2+}}{\rightleftharpoons} \beta\text{-aspartyl phosphate} + \text{ADP}$$

Feedback control of aspartate kinase in bacteria can operate by two mechanisms. Cells may have several isofunctional enzymes which are regulated individually and specifically by one of the end products. In this case, an excess of one of the terminal

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amino acids cannot prevent the biosynthesis of another end product of the same pathway. This type of regulation was discovered in *Escherichia coli*[1,2] and seems to be quite general in Enterobacteriaceae[3]. Furthermore, in *E. coli* K 12 and B, aspartate kinase I is a multifunctional enzyme, carrying also homoserine dehydrogenase activity, similarly subject to repression and inhibition by threonine[4]. In the strain K 12 aspartate kinase II is also multifunctional; the same protein carries a second homoserine dehydrogenase activity, subject to repression by methionine[2].

Other bacteria possess a single aspartate kinase, inhibited by one or more of the end products and subject to a concerted feedback inhibition by two of them. This second mechanism was observed for the first time in *Rhodopseudomonas capsulata*[5] and in *Bacillus polymyxa*[6].

A recent comparative and systematic study[3] on the regulation of the activity of enzymes situated at the branching points of the aspartate pathway showed that the pattern of inhibition is a stable group character among micro-organisms.

Aspartate kinases of different bacteria, namely, purple bacteria[5,7,8], Bacillus species[6,9,10] or others[11,12], which are controlled by concerted feedback inhibition, show definite differences with respect to sensitivity to and repressibility by the allosteric inhibitors.

In order to understand these fundamental differences in regulation properties and the possible altered patterns of feedback inhibition of aspartate kinase depending on physiological conditions of the cell, it would be interesting to compare the chemical structures of these enzymes from different bacteria.

For this reason we started to investigate the aspartate kinase in *Pseudomonas putida*, a very common soil bacterium, belonging to the fluorescent group of aerobic pseudomonads[13]. The aspartate kinase of this species is sensitive to feedback inhibition by either L-lysine or L-threonine alone and is subject to a concerted inhibition by these amino acids[3].

In the present work we report the partial purification, some kinetic properties and a peculiar way of regulation of aspartate kinase in this species.

MATERIAL AND METHODS

Strain

Pseudomonas putida A.3.12. used in this work originated in the culture collection of the Department of Bacteriology and Immunology, University of California, Berkeley, Calif.[13].

Chemicals

All chemicals used were of the highest purity available. The salts, magnesium titriplex, (dipotassium–magnesium salt of ethylenediamine tetraacetic acid), glycerol, hydroxylamine and the amino acids (with the exception of L-aspartic acid which was from Calbiochem) were purchased from E. Merck A.G. (Darmstadt, Germany).

The sodium salt of ATP was a product of Schwarz (France). Tris came from Fluka (Switzerland). DL-β-Aspartyl hydroxamate was purchased from Sigma Chemical Co., DEAE-Sephadex and Sephadex G-200 from Pharmacia (Sweden). Dehydrated nutrient broth came from Difco and Ionagar No. 2 from Oxoid Ltd., G.B. Streptomycin was purchased from Specia (France).

Growth of bacteria

All media were prepared from a basal salt solution of the following final composition (g/l): Na_2HPO_4, 1.47; KH_2PO_4, 0.648; $MgSO_4$, 0.2; $FeSO_4$, 0.001; sodium glutamate, serving as a source of carbon and nitrogen, was sterilized by filtration and added separately at a final concentration of 0.02 M. The pH of the media was about 7.2.

Stock cultures were maintained on nutrient broth agar slants. Liquid cultures were grown in violently aerated flasks at 30°. Growth was determined turbidimetrically in a Spectronic 20 (Bausch and Lomb) colorimeter at 660 nm. Nutrient broth media contained 8 g/l of dehydrated nutrient broth.

Buffer solution

The basic buffer solution used in this work was of the following composition: 20 mM potassium phosphate (pH 7.2), 150 mM KCl, 2 mM magnesium titriplex, 5 mM potassium L-aspartate.

Repression experiments

Cultures with sodium glutamate as the carbon and nitrogen source were stopped when absorbance had reached the value of 0.6. The cultures were divided into two equal parts. One part was immediately centrifuged. To the other part an equal volume of complete fresh medium was added with the amino acid to be tested as repressor and sufficient sodium glutamate to maintain the growth of bacteria during four generation times in the exponential phase.

Enzyme assay

The activity of aspartate kinase was measured as described by STADTMAN *et al.*[1]. The amount of aspartylhydroxamate produced was calculated by using a standard curve, established with synthetic β-DL-aspartic acid hydroxamate.

One unit of enzyme is defined as the amount which produces 1 nmole of aspartylhydroxamate per min under these assay conditions. Specific activity is expressed as units per mg protein.

In some cases enzyme activity is given in arbitrary units, such as $A_{540\ nm} \times 1000$ per 30 min.

Protein determination

Protein was estimated by the biuret method[14] or by its ultraviolet absorption[15].

TABLE I

EFFECT OF DIFFERENT PROTECTING AGENTS ON CATALYTIC ACTIVITY OF ASPARTATE KINASE

Protecting agent added during sonication	*Concn. (mM)*	*Specific activity in crude extracts*
None	—	9.3
L-Lysine + L-threonine	0.5 each	7.6
L-Aspartate	10	14.0
L-Aspartate	5	14.0
β-Mercaptoethanol	10	8.5

RESULTS

Enzyme purification

All purification steps were carried out at 0–4°. At room temperature a slight loss of activity was observed with crude extracts. The most valuable protecting agent proved to be potassium aspartate 5 mM (Table I). As shown, neither β-mercaptoethanol nor lysine *plus* threonine were effective in protecting the catalytic activity of the enzyme during sonication.

Preparation of cell-free extracts

Cells were harvested by centrifugation at 700 × *g* for 10 min at 0°, when the culture approached the end of the exponential phase. The bacteria were washed by centrifugation in the buffer solution. The pellets were stored at −20° until use.

20–30 g of frozen cells were then thawed, suspended in the same buffer solution (1.5 ml of buffer per g of wet weight bacteria) and sonically disrupted for 15 min with an MSE 100-W ultrasonic disintegrator. The probe was prechilled and the samples were cooled in ice during sonic treatment. The broken-cell suspension was centrifuged at 100 000 × *g* for 60 min in a Martin Christ "Omikron" refrigerated ultracentrifuge at 4°.

Protein concentration in this extract was about 45–50 mg/ml, and the specific activity of aspartate kinase was 14–15 units/mg protein. This fraction is hereafter referred to as Fraction I.

Streptomycin precipitation

Most of the nucleic acids were precipitated by addition of streptomycin sulfate, final concn. 3% w/v, to the supernatant with constant stirring for 3 h. The suspension was then centrifuged for 20 min at 15 000 × *g*. Fraction II is the supernatant fraction. This step did not alter the specific activity of the enzyme.

$(NH_4)_2SO_4$ fractionation

Thoroughly ground solid $(NH_4)_2SO_4$ was slowly added to Fraction II with constant stirring to give a saturation of 25%. This suspension was allowed to precipitate overnight (15–16 h). The precipitated material was discarded after centrifugation for 20 min at 15 000 × *g*.

The supernatant was then brought to 48% $(NH_4)_2SO_4$ saturation. The precipitate formed within 2 h was centrifuged as before, resuspended and dialyzed against 10 vol. of buffer for 16 h. After this purification step, the specific activity of the enzyme was about 33–35 units/mg (Fraction III) and the yield was 85–95%. There was no aspartate kinase activity in proteins precipitating above 48%.

DEAE–Sephadex chromatography

The dialyzed fraction was then adsorbed onto a DEAE–Sephadex A-50 column (36 cm × 3.8 cm, 50–100 mg protein per g of dry gel). The proteins were eluted by a linear KCl gradient in the buffer solution. The total volume of the gradient was 2500 ml. The concentration of KCl ranged from 0.15 M to 0.35 M (Fig. 1). This procedure permitted separation of aspartate kinase (eluted between 0.235 M and 0.26 M

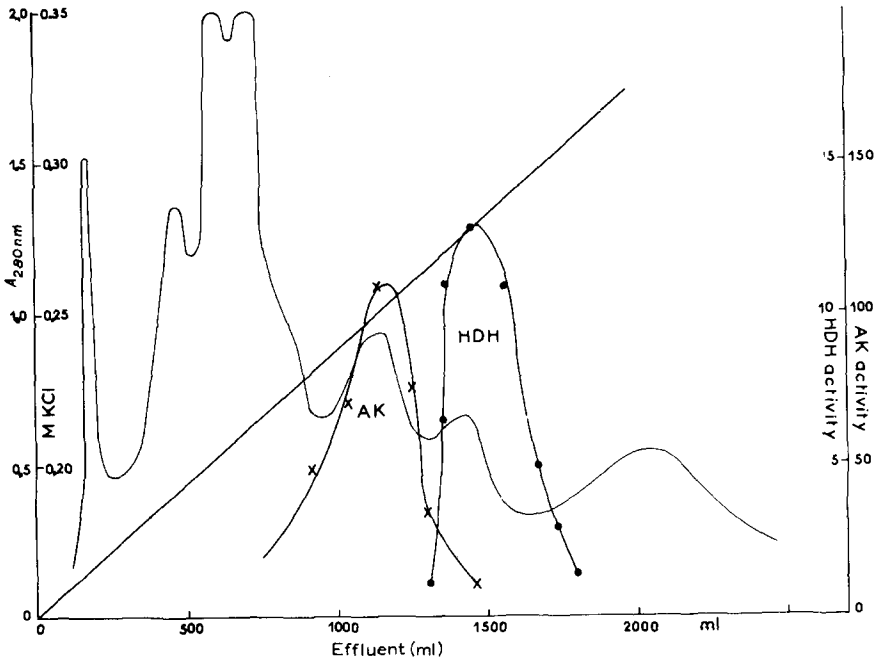

Fig. 1. Separation of the aspartate kinase and the homoserine dehydrogenase (HDH) of *P. putida* by DEAE-Sephadex chromatography. The elution was carried out with a linear gradient of KCl in buffer containing 5 mM L-aspartate. Details are given in the text.

of KCl) and homoserine dehydrogenase (eluted between 0.265 M and 0.30 M of KCl).

The eluates containing the aspartate kinase activity were concentrated either by precipitation with $(NH_4)_2SO_4$ or by slow adsorption of the diluted solution onto a small column (15 cm × 0.5 cm) of DEAE–Sephadex A-50, equilibrated in buffer with 0.12 M KCl. The fixed enzyme was then eluted with a one-column volume of buffer containing 0.28 M of KCl.

Specific activity of aspartate kinase after this step was 394 units/mg, corresponding to an overall 28-fold purification. This fraction (IV) could be stored at 15° for 2–3 months after addition of 20% glycerol (v/v) without loss of activity. Decrease of sensitivity to the inhibitors occurred within a relatively short time, however (Table II).

Properties of the enzyme

Experiments performed with enzyme Fraction I showed a linear relationship between enzyme concentration and the amount of product formation between 0 and 4 nmoles of aspartylhydroxamate. Product formation was also linear with incubation time for at least 45 min at 28°. Optimal pH for the enzyme assay was between 7.9 and 8.4.

Apparent molecular weight was determined by gel filtration on Sephadex G-200 as described by ANDREWS[16]. The markers used were alcohol dehydrogenase from yeast (Worthington, mol. wt. 126 000) and cytochrome *c* from horse heart (Seravac Laboratories Ltd., mol. wt. 12 400). The enzyme solution used was Fraction IV.

TABLE II

THE EFFECT OF STORAGE AT DIFFERENT STAGES OF PURIFICATION ON CATALYTIC ACTIVITY OF ASPARTATE KINASE AND ITS SUSCEPTIBILITY TO FEEDBACK INHIBITION

Experimental details are indicated in the text.

Stage of purification	*Time of storage at −15° (days)*	*Catalytic activity (%)*	*Inhibition by 5 mM L-Lys (%)*	*Inhibition by 100 μM L-Thr (%)*	*Concerted inhibition by 500 μM L-Lys + 10 μM L-Thr (%)*
All fractions*	0	100	82	87	60
Fraction I*	10	100	58	74	60
Fraction III*	25	100	25	53	60
Fraction IV**	25	100	18	26	30

* Enzyme stored in buffer containing 5 mM L-aspartate.
** Enzyme stored in buffer containing 5 mM L-aspartate and 20% of glycerol (v/v).

Buffer containing 5 mM of L-aspartate was used for elution. As shown in Fig. 2, both aspartate kinase and homoserine dehydrogenase are eluted with alcohol dehydrogenase, which suggests a molecular weight of about 126 000.

Enzyme activity during growth

The specific activity of aspartate kinase in crude extracts of cells harvested at various stages in the life cycle was determined. As is shown in Table III, under our growth culture conditions, maximum activity is reached when bacteria are harvested at about $^2/_3$ of the exponential growth phase. Activity begins to decrease before the carbon source is entirely exhausted.

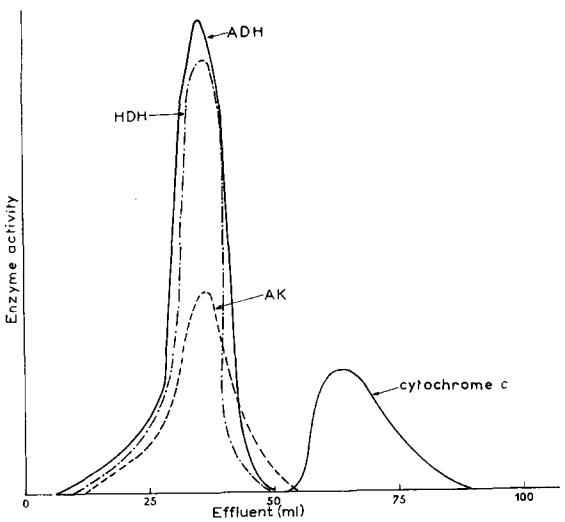

Fig. 2. Elution pattern of aspartate kinase (AK) and homoserine dehydrogenase (HDH) of *P. putida* on Sephadex G-200. Experimental procedure is given in the text. Enzyme activities were measured as follows: aspartate kinase as $A_{540\ nm} \times 1000$ per 30 min of the aspartylhydroxamate complex formed; homoserine dehydrogenase and alcohol dehydrogenase (ADH) as $\triangle A_{340nm}$/min; and cytochrome *c* by extinction at 412 nm.

TABLE III

CATALYTIC ACTIVITY OF ASPARTATE KINASE IN CRUDE EXTRACTS OF *P. putida*. PREPARED AT DIFFERENT STAGES OF GROWTH

Average of three experiments.

A_{660nm}	*Specific activity*
0.48	11
0.58	12
0.63	14
0.75	14
0.85	10
0.90	9
1.18	6.5

Stability studies

Catalytic activity is quite stable at any stage of the purification (See Table II). Enzyme sensitivity to inhibitors is decreasing as its purity is increasing. The loss of sensitivity to lysine occurs first, then threonine sensitivity is affected; finally the extent of concerted feedback inhibition decreases.

Allosteric inhibition of aspartate kinase by end products

As shown in Figs. 3 and 4, in *P. putida* aspartate kinase is inhibited by L-lysine and L-threonine. The enzyme is at least 10 times more sensitive to L-threonine than to L-lysine. To cause half-maximal inhibition, a concentration of 540 μM L-lysine

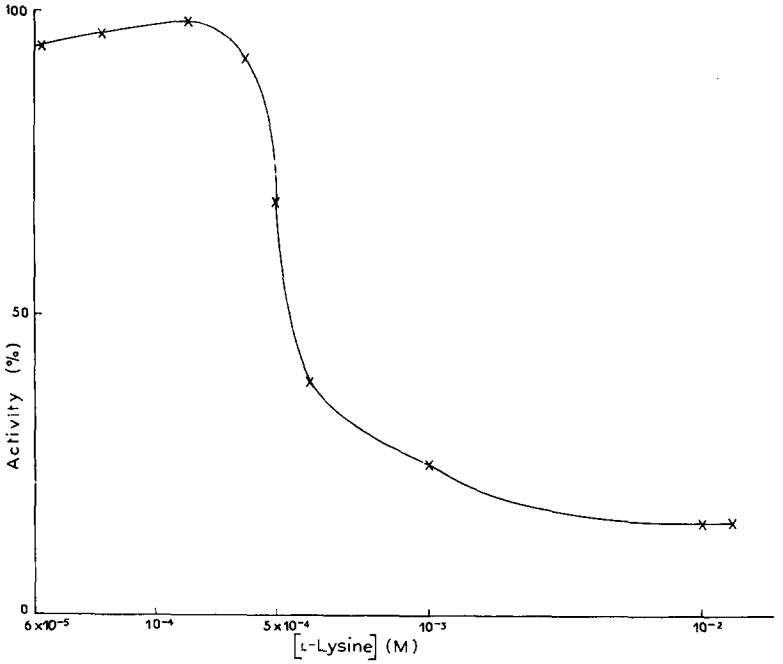

Fig. 3. Inhibition of aspartate kinase activity by L-lysine. The assay was performed with Fraction III (see *Enzyme purification* in the text).

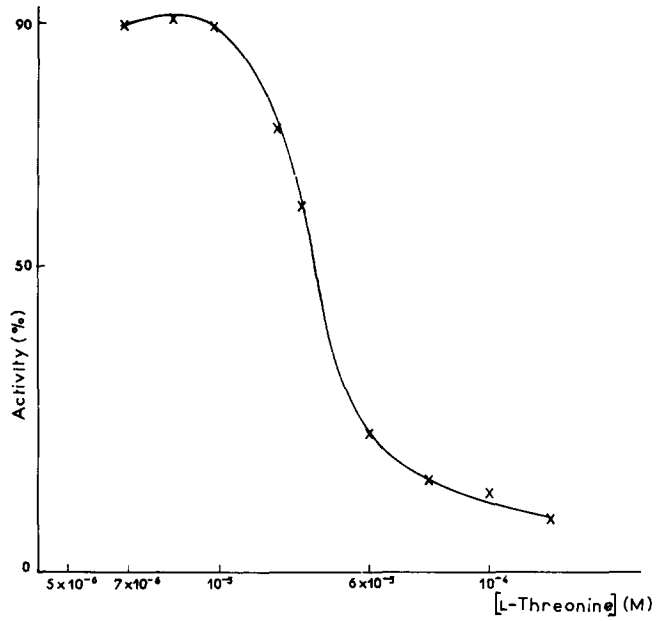

Fig. 4. Inhibition of aspartate kinase activity by L-threonine. The assay was performed with Fraction III (see *Enzyme purification* in the text).

and 42 μM L-threonine, respectively, is necessary. 1 mM L-threonine inhibits enzyme activity to 100%, whereas in the presence of 10 mM L-lysine 10–15% of the enzyme activity is still detectable. Sigmoid-shaped curves obtained with increasing inhibitor concentrations at a constant substrate level indicate cooperativity in the effect of both substances.

Concerted feedback inhibition occurs when L-lysine and L-threonine together are added to the enzyme at low concentrations (Table IV). The inhibition is total when 30 μM L-threonine and 400 μM L-lysine are acting together on aspartate kinase.

TABLE IV

CONCERTED FEEDBACK INHIBITION OF ASPARTATE KINASE BY LOW CONCENTRATIONS OF L-LYSINE *plus* L-THREONINE

The assay was performed with the Fraction III (see *Enzyme purification* in the text). Percentages of inhibition provoked by the addition to the enzyme of a single inhibitor are indicated in parentheses.

Inhibitor conc. (μM)		
L-*Lys*	L-*Thr*	*Concerted inhibition (%)*
100 (2)	10 (10)	30
200 (3)	4 (10)	35
200 (3)	10 (10)	50
300 (3)	2 (10)	60
300 (3)	4 (10)	70
300 (3)	10 (10)	80
400 (8)	30 (27)	100

The two other end products of the branched pathway, L-methionine and L-isoleucine at 10 mM concentration each, do not affect aspartate kinase activity.

Reversibility of inhibition

When the enzyme was preincubated with inhibitory concentrations of either L-lysine or L-threonine and subsequently diluted for assay, full activity was observed. By contrast, when the inhibitor concentration remained the same during the test as before, the enzyme was inhibited by L-lysine or L-threonine or by both in a concerted manner.

Inhibition by structural analogues of L-lysine and L-threonine

None of the L-lysine analogues used significantly inhibited the enzyme activity. Inhibition seems to involve specific requirements concerning the chain length, the absolute configuration and the position of the atoms in the molecule. Thus, D-lysine, L-ornithine, and ε-*N*-formyl-L-lysine are not inhibitory.

10 mM ε-*N*-formyl-L-lysine caused a very slight concerted inhibition in the presence of 5 μM L-threonine. Among the L-threonine analogues, however, DL-*allo*-threonine and α-aminobutyric acid, at respective concentrations 100-fold and 1000-fold higher than L-threonine, inhibited aspartate kinase to a slight extent. These two amino acids and a peptide glycyl-DL-threonine inhibited the aspartate kinase activity in a concerted manner in the presence of 500 μM L-lysine (Table V).

Effect of L-lysine and L-threonine on the heat stability of aspartate kinase

Allosteric enzymes may be protected from heat inactivation by their feedback inhibitors[17]. Therefore, we decided to investigate the influence of L-lysine and

TABLE V

EFFECT OF STRUCTURAL ANALOGUES OF L-LYSINE AND L-THREONINE ON THE CATALYTIC ACTIVITY AND ON THE CONCERTED FEEDBACK INHIBITION OF ASPARTATE KINASE

The experiment was performed with Fraction IV (see *Enzyme purification* in the text).

Analogue	*Concn.*	*Inhibition (%)*	*Inhibition with 5 μM L-Thr (inhibition alone: 10%) (%)*
DL-Lysine	1 mM	32	60
L-Lysine	500 μM	30	80
L-Ornithine	10 mM	0	14
ε-*N*-Formyl-L-lysine	10 mM	1.5	25 with 500 μM L-Lys (inhibition alone: 33%)
DL-Threonine	20 μM	10	65
L-Threonine	10 μM	15	85
L-Serine	1 mM	9	59
DL-Allothreonine	1 mM	18	77
L-α-Amino butyric acid	10 mM	27	64
γ-Amino butyric acid	10 mM	0	10
Glycyl-DL-threonine	10 mM	10	73 with 1 mM DL-allothreonine (inhibition alone: 18%)
L-α-Amino butyric acid	10 mM	27	64

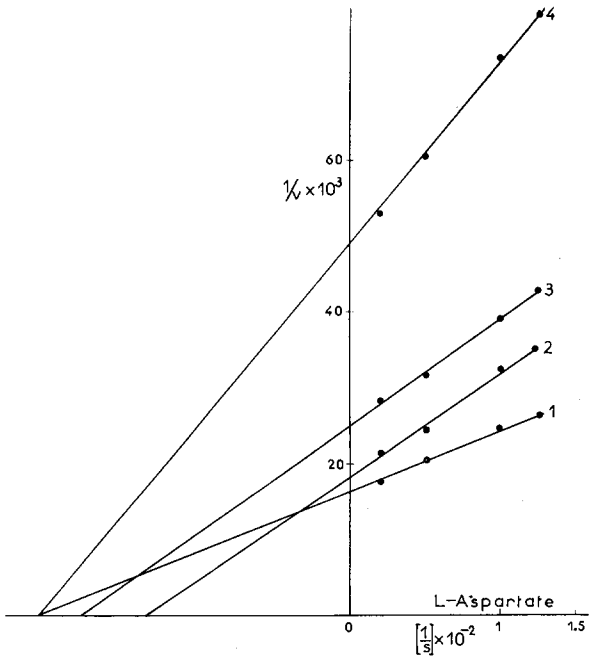

Fig. 5. Lineweaver–Burk plots showing relationship between L-aspartate concentration and aspartate kinase activity inthe absence and presence of feedback inhibitors. Curve 1, enzyme in the absence of inhibitor; Curve 2, enzyme supplemented with L- lysine to 500 μM final concn. Curve 3, enzyme supplemented with L-threonine to 50 μM final concn.; Curve 4, enzyme supplemented with L- lysine and L-threonine to 100 μM and 10 μM final concn., respectively. The enzyme used was from Fraction IV (see *Enzyme purification* in the text).

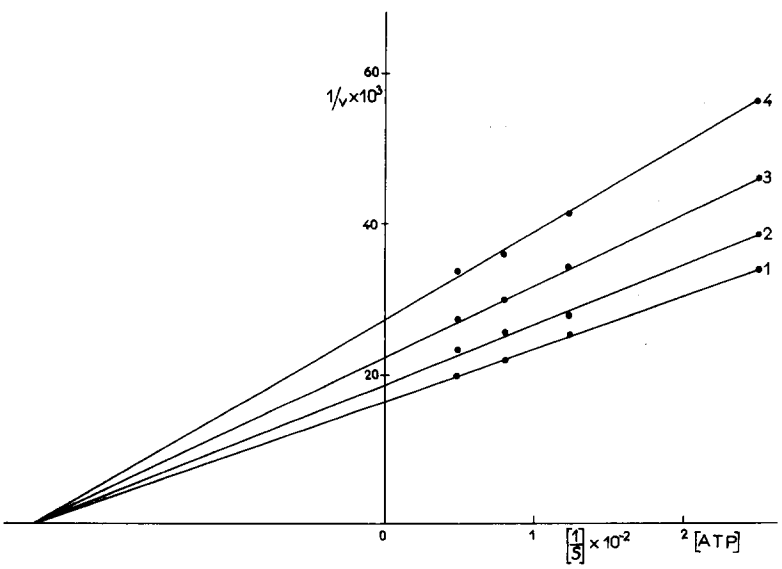

Fig. 6. Analysis of the inhibition of aspartate kinase activity by feedback inhibitors, with ATP as substrate. Curve 1, enzyme without inhibitor; Curve 2, enzyme with 500 μM L-lysine; Curve 3, enzyme with 50 μM L- threonine; Curve 4, enzyme with 100 μM L-lysine + 10 μM L- threonine. The enzyme used was from Fraction IV (see *Enzyme purification* in the text).

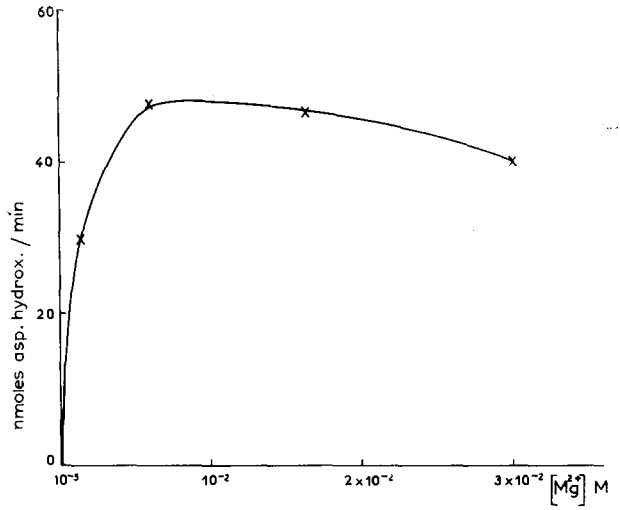

Fig. 7. Relationship between enzyme activity and $[Mg^{2+}]$. Assay mixtures contained 20.7 mM ATP, 10 mM L-aspartate and enzyme, 105 μg protein, after DEAE–Sephadex chromatography (Fraction IV as described in *Enzyme purification* in the text).

L-threonine on the heat sensitivity of aspartate kinase. As demonstrated in Table VI, heating the enzyme for 20 min at 45° leads to a 61% inactivation. The enzyme heated in the presence of 10 mM L-lysine or 100 μM L-threonine or with both at these concentrations lost only 25, 36 and 25% of its catalytic activity, respectively.

Kinetic constants

Figs. 5, 6 and 7 show the variations in activity of the enzyme with subtrates concentrations: L-aspartate (K_m, $4.8 \cdot 10^{-3}$ M), ATP (K_m, $4.35 \cdot 10^{-3}$ M) and Mg^{2+}.

Both allosteric inhibitors acted as mixed competitive–noncompetitive inhibitors with L-aspartate, when added separately to the enzyme. However, concerted inhibition with the same substrate was noncompetitive.

TABLE VI

EFFECT OF HEATING AT 45° ON CATALYTIC ACTIVITY OF ASPARTATE KINASE AND PROTECTION BY FEEDBACK INHIBITORS

The incubation mixture from Fraction III contained: 8 mg of proteins which were heated in 0.5 ml of 1 M Tris–HCl buffer (pH 8.1), in the presence or absence of inhibitors. After incubation, 0.05 ml of mixture containing 800 μg of proteins was taken and immediately cooled in ice.

Inhibitor added	*Inhibitor concn. during*		*Activity (%) Incubation time (min) at 45°*			
	Heating (mM)	*Assay (μM)*	*0*	*10*	*20*	*30*
None	—	—	100	71	39	25
L-Lysine	10	400	90	75	75	68
L-Threonine	0.1	1	97	75	64	60
L-Lysine + L-Threonine	10–0.1	50–1	100	75	75	75

TABLE VII

REPRESSION AND FEEDBACK INHIBITION OF ASPARTATE KINASE OF *P. putida* UNDER DIFFERENT GROWTH CONDITIONS

Enzyme activity is given as $A_{540\ nm} \times 1000$ per 30 min. Experimental conditions are described in MATERIAL AND METHODS. The experiment was performed with Fraction III (see *Enzyme purification in the text*).

Growth supplement	*Concn. of supplement (mM)*	*Generation time*	*Enzyme activity (units/mg protein)*	*Repression %*	*Inhibition (%) by*		
					500 μM L-*Lys*	*10 μM* L-*Thr*	*500 μM* L-*Lys* + *10 μM* L-*Thr*
None	—	1 h 12 min	138	0	37	14	80
L-Lysine	5	4 h 12 min	80	42	12	12	12
L-Threonine	5	2 h 42 min	56	59.5	0	0	0
L-Methionine	5	3 h 30 min	40	71	0	0	0

In the presence of lysine, threonine or both, inhibition was always noncompetitive with ATP as substrate.

Regulation by repression of enzyme synthesis

As indicated in Table VII aspartate kinase synthesis was repressed by the end products.

Repression was greatest with 5 mM L-methionine in the culture (71%).

The most interesting feature of the regulation was that enzyme synthesis was not only repressed when cells were growing in the presence of the inhibitors, but that the enzyme synthesized under these conditions had a completely altered sensitivity to lysine or threonine or to their concerted action.

DISCUSSION

Aspartate kinase, the first enzyme in a branched biosynthetic pathway leading from aspartate to lysine, methionine, threonine and to isoleucine, is in *P. putida* subject to a dual control by its end products, feedback inhibition of its activity and repression of its synthesis.

Only one aspartate kinase appears to be present in this bacteria, this is indicated by the inhibition curves; the activity of the enzyme is quite totally inhibited by the end products (lysine or threonine) at high concentration and at much lower concentration when they act together. In addition, neither $(NH_4)_2SO_4$ fractionation nor DEAE–Sephadex chromatography of the cell-free extract revealed the presence of more than one aspartate kinase. In *P. putida*, unlike *E. coli*, there is no physical association between aspartate kinase and homoserine dehydrogenase.

In branched biosynthetic pathways a special regulatory control mechanism has to be operative in order to permit the synthesis of end products which are not present in excess in the cell. This regulatory function can be achieved by the presence in the cell of several isofunctional enzymes, regulated independently. This was shown to be the case in *E. coli*[2,4,18] as well as in other Enterobacteriaceae[3,19,20]. When only one enzyme is present in the cell as in *P. putida*, the regulatory function has to be obtained

in a different way. The single aspartate kinase of *P. putida* is not inhibited by all the end products situated on this pathway. Furthermore, repression by one end product is never complete.

The inhibition of aspartate kinase seems to be very specific. None of the structural analogues of L-lysine or L-threonine tested individually gave an inhibition comparable to these two amino acids. Concerted feedback inhibition could, however, be obtained by higher concentrations of some analogues.

During storage, the enzyme gradually loses its faculty to be inhibited by the end products. Sensitivity to lysine is lost first, and when sensitivity to threonine is decreasing, the enzyme can still be fully inhibited in a concerted manner by these amino acids.

The fact that neither L-lysine nor L-threonine are strict competitive inhibitors with either substrate (aspartate, ATP) suggests that these amino acids do not bind at the substrate site. The concerted relationship of the two inhibitors indicates that both do not bind at the same site. L-Lysine and L-threonine, respectively, behave as mixed competitive–noncompetitive inhibitors of aspartate kinase with L-aspartate as substrate. A mixture of the two amino acids, however, inhibits the enzyme noncompetitively.

Aspartate kinase synthesis is severely but not entirely repressed when *P. putida* is growing on culture media containing either lysine, threonine or methionine. The enzyme synthesized under these conditions is different from the "normal" enzyme with respect to its sensitivity to feedback inhibitors.

This result, together with the fact that repression is never total, appears as a sort of physiological self-defensive mechanism for this cell, which has no isofunctional enzymes. It may in this way prevent biosynthetic disorders, even when one of the endproducts is in excess in the cell.

A similar observation was described by KINGDON AND STADTMAN[21,22] concerning glutamine synthetase of *E. coli* and by GRAY AND BERNLOHR[23] about aspartate kinase of *Bacillus licheniformis*, each having entirely altered sensitivity to feedback inhibitors, when synthesized in their presence.

Investigation of this phenomenon in other bacteria might reveal whether this type of control mechanism is a general one in cells not possessing isofunctional enzymes. Furthermore, a detailed study of the enzyme structure seems to be warranted in order to explain the great difference in sensitivity to inhibitors of the enzyme synthesized under normal conditions and under endproduct repression.

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