ASPARTOKINASE OF LYSINE EXCRETING AND NON-EXCRETING STRAIN OF *BACILLUS MEGATERIUM*

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ABSTRACT

The lysine non-excreting *B. megaterium* 7581 showed a single aspartokinase, repressed and inhibited synergistically by threonine plus lysine. The lysine excreting *B. megaterium* CII 19 showed two aspartokinase(s), one represed and inhibited by threonine and the other repressed by methionine but inhibited by methionine plus lysine. The lysine excretor has aspartokinase activity considerably higher than the lysine non-excreting strain and the maximal activity persists for a long time.

INTRODUCTION

Aspartokinase (ATP: L aspartate phosphotransferase, EC 2.7.2.4.) is the first enzyme of the series of reactions leading to the biosynthesis of the aspartate family of amino acids in bacteria. Bacteria show considerable diversity[1] in the feedback regulation of aspartokinase. In *Escherichia coli*[2], *Salmonella typhimurium*[3] and some other Enterobacteriaceae[4] there are three different aspartokinases repressed and or inhibited by threonine, methionine and lysine, though the predominant aspartokinase varies from one strain to another. In *Bacillus polymyxa*[5], *Rhodopseudomonas capsulatus*[6], pseudomonads[7] and lysine overproducing microbes[8], there exists a single aspartokinase and their regulation is achieved either by a multivalent or by synergistic inhibition involving both lysine and threonine.

The present communication reports the activity of aspartokinase under different phases of growth in two biotin requiring strains of *Bacillus megaterium*, one excretes lysine (CII 19) and the other (7581) does not. The pattern of regulation of their aspartokinase has also been compared.

MATERIALS AND METHODS

B. megaterium CII 19, a lysine excretor, was isolated from Indian soil[9] by its ability to cross-feed lysine auxotrophs of *E. coli* K12. *B. megaterium* 7581 was procured from Dr P. J. White, Department of microbiology, Sheffield University, U.K. The strains were maintained on the agar slopes of medium A–1[9] as modified by Chatterjee and White[10], supplemented with 1 μg 1^{-1} biotin. The organisms were grown in shaken flasks in the same liquid medium on a rotary shaker at 30°C. Growth was measured turbidimetrically and extracellular lysine estimated colorimetrically[11]. An aqueous suspension of bacteria from a 24 hr old culture on agar slope was used (10^6 cells ml^{-1}, 2% v/v) to inoculate the flasks. The organisms were harvested by centrifugation at 10000 g for 45 min and washed twice with sodium phosphate buffer at pH 7.5. The cell-free extracts were prepared from cells frozen overnight by crushing them in sand with half its volume of triethanolamine-HCl buffer at pH 8.0 and the extract was centrifuged at 10000 g for 20 min at 2°C. The extract was dialyzed overnight using the same buffer and its protein content was measured[12]. Aspartokinase was assayed[13] by measuring at 540 mμ the aspartohydroxamate formed.

RESULTS AND DISCUSSION

Aspartokinase activity of the lysine overproducing strain CII 19 increased gradually during the growth (table 1). The activity showed a steady rise during 18 to 24 hr and reached a maximum at the end of the exponential phase. The major bulk of lysine accumulated during 30 to 42 hr. This high level of aspartokinase activity was maintained till 48 hr. The lysine non-excreting strain 7581 has aspartokinase activity considerably lower than the CII 19. Aspartokinase activity of the strain 7581 was also maximum at the end of the exponential phase of growth but it reduced to about 50% during the next 24 hr. It is well known[14] that in some bacilli the activity of aspartokinase rapidly reduced in the post-log phase, but in some other *Bacillus* species

the activity was maximum only a few hours after the exponential phase.

The pattern of regulation of the aspartokinase of the two strains was compared by adding lysine, methionine and threonine, singly or in combinations to the growth medium (table 2) and in the assay system (table 3). Methionine, when added to the growth medium of CII 19 partly (44%), repressed the aspartokinase and the residual activity was insensitive to inhibition by methionine or lysine, but was inhibited strongly (85%) by 1 mM threonine in the assay mixture. Methionine and lysine when added together in the assay mixture partly (15%) inhibited the residual activity present in organisms grown with methionine. Threonine also partly repressed (46%) aspartokinase and the residual activity was insensitive to inhibition by threonine, methionine or lysine alone, but completely inhibited when lysine and methionine were added together in the assay system. Methionine and threonine when added together in the assay mixture completely show a little aspartokinase activity which was insensitive to inhibition by methionine, threonine or lysine alone but was completely inhibited by methionine plus lysine. When added to the assay system (table 3) threonine alone inhibited 51% of the aspartokinase of CII 19. Neither lysine nor methionine alone was inhibitory; when they were added together in the assay mixture completely inhibited aspartokinase activity.

When added to the growth medium of the lysine non-excreting strain 7581, methionine does not repress the aspartokinase. Lysine or threonine slightly repressed (table 2) the aspartokinase and the residual activity in both the cases were not sensitive to inhibition by lysine or threonine alone, but completely inhibited when lysine and threonine

Table 1 *Aspartokinase activity during growth of the two strains of Bacillus megaterium*

	B. megaterium 7581		*B. megaterium* CII19		
Time (hr)	Growth[a]	Aspartokinase[b]	Growth	Extracelluar[c] lysine	Aspartokinase
6	0.6	25	0.5	–	40
12	1.1	32	1.0	–	44
18	2.3	36	2.0	0.4	50
24	2.9	40	2.8	0.8	56
30	3.2	30	3.0	2.0	52
36	3.2	25	3.0	2.9	52
42	3.2	23	3.0	3.6	52
48	3.2	20	3.0	3.9	52
54	3.2	20	3.0	3.8	50

[a]OD in EEL units; [b]Units, nmol of aspartohydroxamate formed min^{-1} mg^{-1} protein; [c]mg/ml.

Table 2 *Activity of aspartokinase in extracts of the two strains of B. megaterium after growth with various additions in the medium*

Addition(s) (all 1 mM)	Aspartokinase activity	
	B. megaterium CII19	*B. megaterium* 7581
None	100 (56)	100 (40)
L-Methionine	56[a]	102
L-Threonine	54[b]	80[d]
L-Lysine	100	82[d]
L-Methionine+L-Threonine	15[c]	80[d]
L-Threonine+L-Lysine	55	4[d]
L-Methionine+L-Lysine	55	84[d]
L-Methionine+L-Threonine +L-Lysine	Undetectable	Undetectable

[a]Activity slightly (15%) inhibited by L-methionine plus L-lysine and strikingly (85%) by threonine in the assay system.
[b]Activity completely inhibited by lysine plus methionine in the assay system.
[c]Activity completely inhibited by L-lysine plus L-methionine in the assay system.
[d]Activity completely inhibited by L-lysine plus threonine in the assay system.

Table 3 *Activity of aspartokinase in extracts of the two strains of B. megaterium with various additions in the assay mixture*

Addition(s) (all 1 mM)	Aspartokinase activity	
	B. megaterium CII19	*B. megaterium* 7581
None	100 (56)	100 (40)
L-Methionine	102	102
L-Threonine	49[a]	85[a]
L-Lysine	100	80[a]
L-Methionine+L-Lysine	52[a]	80[a]
L-Threonine+L-Lysine	47	Undetectable
L-Methionine+L-Threonine	47	85[a]
L-Methionine+L-Threonine +L-Lysine	Undetectable	Undetectable

[a]No further inhibition at 5 mM concentration.

were added together in the assay system. Lysine and threonine when added together in the medium the organism showed very poor growth and a very little aspartokinase activity which was undetectable when lysine and threonine were added together in the assay mixture. Lysine and threonine when added in the assay mixture (table 3) slightly inhibited aspartokinase, whereas methionine does not. Aspartokinase activity was completely inhibited when lysine and threonine were added together in the assay mixture.

Repression and inhibition of aspartokinase have been studied intensively in many organisms[4]. The lysine non-excreting strain 7581 seems to have a single aspartokinase which is repressed and inhibited synergistically by lysine and threonine. A strikingly similar pattern of regulation of aspartokinase has been reported in *B. polymyxa*[5], *B. licheniformis*[15], and a number of other *Bacillus* species[5]. *B. subtilis*[16], however, has two aspartokinases; aspartokinase I, the major one is inhibited by diaminopimelate and aspartokinase II inhibited by lysine and threonine in concert. The lysine excreting strain CII 19 shows two aspartokinases; one repressed and inhibited by threonine and the other repressed by methionine but inhibited by methionine and lysine in concert. Other lysine-producing organisms like *Brevibacterium flavum*[17] and *Corynebacterium glutamium*[18] have a single aspartokinase repressed and inhibited by lysine and threonine in a concerted manner. The lysine excreting strain CII 19, has aspartokinase(s) with two important properties, apart from its pattern of regulation; strikingly higher activity that persists long after the exponential phase and insensitivity to lysine, that made it a potent lysine excretor.

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NEWS

COMPUTER 'SEES' THROUGH EARTH

A computer-aided mathematical model of earth crust has been created at the USSR Academy of Sciences' Institute of Geo-chemistry and Analytical Chemistry. It will help understand the complex processes of crust formation.

A special role in the formation of the crust and its components was played by the processes which were triggered by magma eruptions from the mantle lying at depths 50–70 km in strike. Part of the magma rose to the surface as lava, while the bulk of it formed interesting natural objects—intrusive lenses—at a depth of 3–5 km. These lenses were cavities filled with molten magma, which can be seen as the initial material for forming the earth crust.

The lenses were from 100–300 m thick and the volume of cavities up to several cubic km. While on the surface, it took the lava years or scores of years to cool down, depending on the layer thickness, inside the lenses the process lasted even centuries. During that time, the chemical components of the smelt became separated during crystallization.

Natural cataclysms brought these rocks to the surface in many parts of our planet. Knowing the chemical composition of the samples taken from several ex-intrusive lenses found in the area between the Yenisei and Lena rivers and in the territory of the GDR and USA, the researchers have described mathematically the processes which had led to their formation. Correctness of these calculations had been proved by the computer. The mathematical model suggests new methods of ores prospecting. (*Soviet features*, Vol. XXV, No. 143, September 18, 1986; Information Department, USSR Embassy in India, P. B. 241, New Delhi 110 001).

PAMIR MOUNTAIN CHAINS DRIFT

Laser-aided research by scientists in Tajikistan Soviet Central Asian Republic has revealed that two mountain ranges in the Pamirs have moved 50 cm closer to each other over the last thirty years. By regularly measuring with the light ray the distance between the Karateghinski and the Peter I ridges, they noticed a previously unknown phenomenon. It has become apparent that the two giants alternatively draw closer and move off.

According to the scientists, the mountain drift is caused by tectonic processes deep inside the earth and is not catastrophic. This is one of the previously unknown natural phenomena. Most researchers are inclined to think that two giant 'floating' platforms on whose surface many Asian states are situated, lie close to each other in this region. Experiments corroborate this hypothesis, yet many questions still remain unanswered. (*Soviet features*, Vol. XXV, No. 141, September 15, 1986; Information Department, USSR Embassy in India, P. B. No. 241, New Delhi 110 001).