ASPARTOKINASE OF *MICROCOCCUS LUTEUS*
'Feedback-stimulation' by methionine

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1. Introduction

The enzyme aspartokinase (ATP, L-aspartate 4-phosphotransferase, EC 2.7.2.4) catalyzes the first reaction in the biosynthetic pathway leading to L-lysine, L-isoleucine, L-threonine, L-methionine and α-ε-diaminapimelic acid (Dap). Generally, the aspartokinases of microorganisms are feedback-inhibited and repressed by one or more amino acids of the aspartate family. Recently, we reported the activation of the aspartokinase of *Myxococcus xanthus* by methionine and isoleucine [1,2]. This activation was especially interesting since these two amino acids, although members of the aspartate family, are required for the growth of *M. xanthus*. This led us to postulate that *M. xanthus* lost its ability to synthesize these amino acids by retrograde evolution, but retained a vestige of its former control mechanism. Instead of the characteristic inhibition by the end-product on the first enzyme of the biosynthetic pathway, isoleucine and methionine activated both aspartokinase isoenzymes of *M. xanthus* [1,3]. This phenomenon was referred to as 'feedback-stimulation'. The literature contains one example, *Rhodospirillum rubrum*, of an aspartokinase activity which is stimulated by non-required amino acids [4]. In order to examine whether or not the mechanism of 'feedback-stimulation' extended beyond the myxobacteria, the aspartokinase of a methionine-requiring *Micrococcus luteus* (*lysodeikticus*) was investigated.

† Deceased

2. Materials and methods

An 800 ml culture of *M. luteus* ATCC 4698 (Fleming) was grown to the late exponential-phase at 32°C with vigorous aeration in synthetic medium [5,6]. Cells were harvested by centrifugation and washed twice with cold 0.02 M potassium phosphate buffer, pH 7.5, containing 0.03 M 2-mercaptoethanol. The cells were resuspended in the above buffer and disrupted by three 30 s exposures to sonic oscillation with a Branson B12 sonifier (setting 5), maintaining an extract temperature below 5°C. The supernatant fluid after centrifugation at 34 000 × *g* for 30 min is referred to as the crude extract. The supernatant was further fractionated by addition of a saturated solution of ammonium sulfate, maintaining pH 7.0. Protein precipitating at 30% saturation with ammonium sulfate contained no enzyme activity and was removed by centrifugation (16 000 × *g* for 10 min). To the supernatant fluid, addition ammonium sulfate was added to yield a 70% saturated solution. After centrifugation as above, the pellet was suspended in 0.02 M potassium phosphate buffer, pH 7.5, containing 0.03 M 2-mercaptoethanol.

Aspartokinase activity was determined by the hydroxamate procedure [7]. The reaction mixture contained in 1.0 ml: 10 mM ATP, 30 mM L-aspartate, 10 mM 2-mercaptoethanol, 100 mM Tris (hydroxymethyl aminomethan–HCl) buffer, pH 8.1, 5 mM $MgSO_4$, 800 mM NH_2OH. KCl (prepared by neutralization of NH_2OH–HCl with KOH) and 0.1–1.0 mg crude enzyme protein. Specific activities are expressed as nmol hydroxamate produced/min/mg protein.

Protein was determined by the method of Lowry et al. [8].

3. Results and discussion

Crude extracts of *M. luteus* that had been grown in synthetic medium [5,6] containing 20 μg DL-methionine/ml contained high specific activities of aspartokinase (fig.1, table 1). Preliminary experiments indicated that optimum activity was obtained with 30 mM aspartate, 10 mM ATP and 5 mM $MgSO_4$. Enzyme activity was linear with time for 40 min and protein concentration (crude extract) up to 10 mg/ml.

L-Threonine and L-lysine each inhibited the aspartokinase only 10–15%, whereas a mixture of the two amino acids inhibited the activity 85–90%. L-Isoleucine and meso-Dap, as well as L-phenylalanine, L-tryptophan, L-histidine, L-glycine and L-alanine had no significant effect on the aspartokinase of *M. luteus*. L-Methionine both stimulated the enzyme (60–80%) and overcame the inhibitory effect of lysine and threonine. In each case about 50% of the effect of the amino acid was observed at 0.3–0.4 mM. Similar results were seen with the crude extract and 30–70% ammonium sulfate fraction.

The activation of *M. luteus* aspartokinase by methionine provides additional evidence for the concept of 'feedback-stimulation'. Aspartokinase

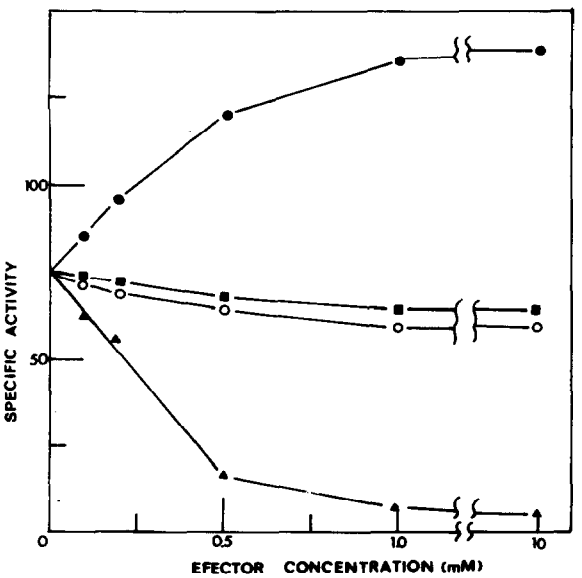

Fig.1. Effect of amino acids on the aspartokinase activity of *M. luteus*. Activities were measured in the presence of varying amounts of L-methionine (●), L-lysine (■), L-threonine (○) and L-lysine plus L-threonine (▲).

activity has been examined in only two bacterial species in which there is a natural requirement for one or more members of the aspartate family, the Gram-negative *M. xanthus* and the Gram-positive *M. luteus*. In both examples the required amino acids

Table 1
Effect of certain amino acids on the aspartokinase of *M. luteus*

Additions (1 mM)	Specific activity[a]	
	Crude extract	$(NH_4)_2SO_4$ Fraction
None	75	131
L-Methionine	137	206
L-Isoleucine	72	125
L-Threonine	65	117
L-Lysine	63	113
Meso-Dap	76	128
L-Methionine + L-threonine	107	129
L-Methionine + L-lysine	110	132
L-Methionine + L-isoleucine	135	205
L-Threonine + L-lysine	11	13
L-Threonine + L-lysine + L-methionine	71	127

[a] The preparation and assay of the crude extract and 30–70% ammonium sulfate precipitate fraction for aspartokinase activity was as described in Materials and methods

specifically stimulated the aspartokinase activity. In general, it is reasonable to assume that following mutation to loss of synthetic ability, there would occur strong selective pressure for a second wave of regulator mutations. In this particular case, 'feedback-stimulation' would have the selective advantage of insuring adequate synthesis of cell-wall precursors in rich media and reducing its production during starvation of the required amino acid. Mutation and end-product activation has previously been reported in the phenylalanine pathway in *Bacillus subtilis* [9].

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