Lysine-Enhanced Threonine-Inhibition of Bacterial Aspartokinase: Concerted or Synergistic Feedback Inhibition?

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The concept of concerted and synergistic feedback inhibition of the regulatory enzyme of a branched biosynthetic pathway is critically discussed on the basis of data obtained with the threonine-lysine-sensitive aspartokinase of *Rhodospirillum rubrum*. It is proposed that only one name (concerted feedback) should be used to specify allosteric feedback inhibitions where at least two endproducts of the branched pathway are required for complete inhibition of the regulatory enzyme.

Introduction

In branched biosynthetic pathways in which one common precursor is converted to several metabolites, simple endproduct repression of enzyme synthesis or feedback inhibition of enzyme activity is often not sufficient to control the early enzymes of the pathway. According to Stadtman [1], six different control mechanisms are realized in branched biosynthetic pathways: (a) enzyme multiplicity, (b) multivalent enzyme repression, (c) sequential feedback inhibition, (d) concerted feedback inhibition, (e) synergistic feedback inhibition, and (f) cumulative feedback inhibition.

The concepts of enzyme multiplicity, multivalent repression and sequential feedback are experimentally well documented. On the other hand, the differences between the concepts of "concerted", "synergistic" and "cumulative" feedback inhibition are much less convincing. Thus, the "concerted" and "synergistic" feedbacks have in common that one single endproduct of the branched pathway is not sufficient to cause complete inhibition of the corresponding regulatory enzyme. In the concerted type of feedback, neither of the endproducts alone is able to bring about a measurable inhibition of the target enzyme, while, in the synergistic case, each endproduct should have a measurable effect. Both mechanisms call for at least two endproducts to effect complete inhibition. Although, in an extended study on feedback patterns of aspartokinase in coliform bacteria and pseudomonads, Cohen *et al.* [2] found that the extent of "concerted" feedback inhibition of aspartokinase strongly depended on the concentrations of the two feedback modifiers, threonine and lysine, the significance of the two terms "concerted" and "synergistic" feedback was not critically discussed.

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Results and Discussion

Contrary to coliform bacteria, the majority of gram negative as well as gram positive bacteria contain only one single aspartokinase whose activity is regulated in a complex way such that two endproducts of the branched pathway, namely L-threonine and L-lysine, are necessary for complete allosteric feedback inhibition. This type of feedback inhibition was first described for the aspartokinase of the phototrophic bacterium, *Rhodopseudomonas capsulata* [3], and was called "concerted feedback inhibition". Parallel to the studies on aspartokinase regulation in phototrophic bacteria, investigations on the feedback control of the key enzyme of purine nucleotide biosynthesis, glutamine phosphoribosylpyrophosphate amidotransferase, revealed a similar

Table I. Amplification of L-threonine inhibition of aspartokinase from *Rhodospirillum rubrum* by L-lysine.

Additions to basic reaction mixture	Aspartokinase activity (nmol/min · mg protein)
No.	60 ± 2
1 mM L-lysine	60 ± 2
5 mM L-lysine	60 ± 2
10 mM L-lysine	52 ± 2
0.5 mM L-threonine	58 ± 2
5 mM L-threonine	21 ± 1
10 mM L-threonine	13 ± 1
5 mM L-lysine plus 0.5 mM L-threonine	30 ± 2
5 mM L-lysine plus 5 mM L-threonine	2 ± 1

R. rubrum strain S1 was grown photosynthetically in a malate-$(NH_4)_2SO_4$-medium supplemented with biotin [5]. The cells were harvested in the late exponential growth phase, resuspended in 20 mM K-phosphate, pH 7.2, supplemented with 150 mM KCl, 2 mM EDTA, 2 mM $MgSO_4$, 1 mM L-threonine, 0.5 mM L-lysine, and disrupted by ultrasonic treatment. Aspartokinase was precipitated from the 15,000 × *g*-supernatant of the homogenate by $(NH_4)_2SO_4$ (35–50% saturation), and dissolved in the buffer quoted before. Its activity was assayed as described in ref. [3].

feedback pattern. Here, combinations of nucleotides, for example AMP plus GMP, were found to be necessary for complete inhibition [4], and the term "synergistic" was used to describe such regulatory behaviour. Compared with the description of the "concerted" model described by Datta and Gest [3], the "synergistic" model differs from the "concerted" only in that *each* of the two endproducts has a *significant* inhibitory effect on the target enzyme.

It can easily be shown that by using the appropriate effector concentrations, one biosynthetic enzyme may exhibit allosteric feedback responses of both the concerted and the synergistic type. The data of Table I show that the aspartokinase of the phototrophic bacterium, *Rhodospirillum rubrum*, is only weakly inhibited by L-lysine alone, but moderately sensitive to inhibition by L-threonine. In the presence of 5 mM L-lysine, the inhibition by L-threonine is significantly enhanced. Since the inhibitory effects obtained by either 0.5 mM L-threonine or 5 mM L-lysine are neglectable, the term "concerted feedback" would be appropriate if one would restrict oneself to these concentrations. However, if one would select individual endproduct concentrations of 5 (threonine) and 10 (lysine) mM, the overall feedback response would be described by the term "synergistic". Thus, the data obtained with the *R. rubrum* aspartokinase show that the regulatory enzyme of a branched biosynthetic pathway exhibits feedback patterns of both the "concerted" and "synergistic" type. I propose, therefore, that only one term (concerted feedback) should be used to describe the behaviour of such allosteric enzymes.

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