BBA 61178

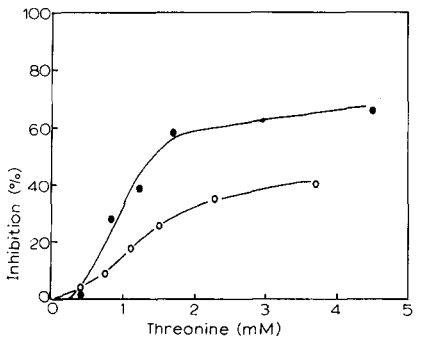
Concerted feedback inhibition of aspartokinase from *Bacillus stearothermophilus*

One of the common properties of many endproduct-inhibited enzymes of mesophilic bacteria is that they can be desensitized to endproduct regulation by heat treatment at 50° or above *in vitro*[1-4]. At elevated temperatures the conformations of these allosteric enzymes are presumably altered such that the enzymes are no longer sensitive to endproduct regulation. Since thermophilic bacteria grow readily at these elevated temperatures, it was of interest to investigate the nature of allosteric regulation in these organisms. This report is concerned with the regulatory properties of aspartokinase (EC 2.7.2.4) from the thermophilic bacterium *Bacillus stearothermophilus*. This enzyme catalyzes the first reaction in the aspartate pathway leading to lysine, threonine, methionine and isoleucine[5]:

ATP + aspartate → aspartyl phosphate + ADP

B. stearothermophilus strain 1503-4R (obtained through the courtesy of N. WELKER) was grown in a tryptone–yeast extract medium at 55° as previously described[6]. Crude extracts were prepared following sonic oscillation of the cells (1.0 g of cells suspended in 4.0 ml 0.02 M Tris buffer (pH 7.4)–5 mM mercaptoethanol) for 1 min at 8 mA utilizing a Branson Sonifier. The enzyme was partially purified following treatment with streptomycin sulfate and ammonium sulfate fractionation. The ammonium sulfate fraction precipitating between 45 and 60% of saturation served as the source of aspartokinase in all of the following experiments. The enzyme was assayed according to the hydroxamate assay of STADTMAN *et al.*[7].

Aspartokinase from the thermophile was shown to be sensitive to feedback inhibition by two of its endproducts lysine and threonine, over a wide temperature range including its temperature optimum of 55° (Figs. 1 and 2). Several other po-



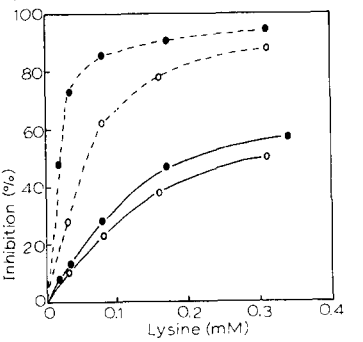


Fig. 1. Feedback inhibition of aspartokinase activity by threonine at 37° and 55°. ●—●, inhibition by threonine at 37°; ○—○, inhibition by threonine at 55°. The standard assay mixture consisted of the following components: 87 mM Tris (pH 8.0), 1.5 mM $MgCl_2$, 0.70 M NH_2OH (pH 8.0), 0.70 M KCl, 17.4 mM aspartate, 8.7 mM ATP, enzyme, and water in a total volume of 1.15 ml.

Fig. 2. Feedback inhibition by lysine and concerted feedback inhibition by both lysine and threonine at 37° and 55°. ●—●, inhibition by lysine alone at 37°; ●— — —●, inhibition by lysine and threonine (0.4 mM) at 37°; ○—○, inhibition by lysine alone at 55°; ○— — —○, inhibition by lysine and threonine (0.4 mM) at 55°.

tential feedback inhibitors, methionine, isoleucine, and homoserine, had little effect at either 37° or 55°. A comparison of Figs. 1 and 2 indicates that the enzyme is much more sensitive to lysine inhibition than to threonine inhibition at either 37° or 55°. The enzyme appears to be less sensitive to threonine inhibition when assayed at 55° than at 37°, while the sensitivity to lysine inhibition is only slightly decreased at the higher temperature. Heating the enzyme alone at 55° for 10 min had no effect on either activity or feedback sensitivity when measured at 37°. Furthermore, like the aspartokinases from two other species of Bacillus[8,9], the thermophile enzyme is inhibited by both lysine and threonine together in a concerted manner (Fig. 2). It was observed that the inhibitions produced by combinations of lysine and threonine were much greater than the sum of the inhibitions produced by each amino acid alone. The almost complete abolition of aspartokinase activity by the concerted action of lysine and threonine indicates that the activity is due to only one enzyme. The concerted effect of both amino acids at low lysine concentrations (< 0.10 mM) appeared to be greater at 37° than at 55°. Thus, the sensitivity of the thermophile aspartokinase to lysine, threonine, and the concerted action of both amino acids appears to be diminished in varying degrees when assayed at elevated temperatures.

Lysine appeared to act as a mixed competitive–noncompetitive inhibitor of aspartokinase activity at both 37° and 55° (Fig. 3) affecting both the v_{max} of the reaction and the K_m for aspartate. The addition of threonine did not change the type of inhibition although the K_m for aspartate was further increased. Lysine acted as a noncompetitive inhibitor with respect to the other substrate, ATP. Threonine alone also appeared to act as a mixed competitive–noncompetitive inhibitor with respect to aspartate and was noncompetitive with respect to ATP.

The observation that the sensitivity to threonine inhibition decreases while enzymatic activity increases as the assay temperature is raised, together with the absence of a strict competitive relationship with either substrate, suggests that threonine does not bind at a substrate site. Furthermore, since lysine also does not

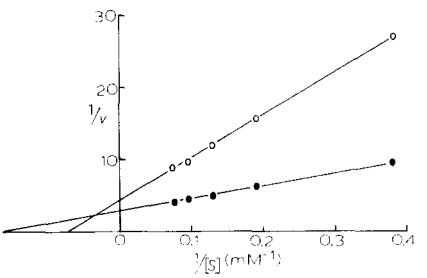


Fig. 3. Effect of lysine on the K_m for aspartate at 55°. ●—●, activity in the absence of lysine; ○—○, activity in the presence of 0.35 mM lysine.

act as a strict competitive inhibitor with respect to either substrate and is effective at relatively low concentrations compared to the substrates, it appears that lysine does not bind at a substrate site either. Finally, the concerted relationship between the two inhibitors indicates that both do not bind to the same site. These results suggest that the aspartokinase of *B. stearothermophilus* possesses two separate regulatory sites distinct from the substrate sites, as has been postulated for the asparto-

kinase of *Bacillus polymyxa*[9]. More rigorous proof of this hypothesis will be sought utilizing binding studies on highly purified enzyme preparations. The present observations also indicate that the regulatory enzymes of thermophilic bacteria are able to maintain feedback sensitivity even at temperatures as high as 55°. It will also be of interest to determine the chemical and physical bases for such maintenance at elevated temperatures.

This investigation was supported by a grant from the Northwestern University Dental School Fluid Research Fund and by grant AM-12132-01 from the National Institutes of Health.

Department of Microbiology,
Northwestern University Medical School,
Chicago, Ill. 60611 (U.S.A.)

HOWARD K. KURAMITSU

1 J. C. GERHART AND A. B. PARDEE, *J. Biol. Chem.*, 237 (1962) 891.
2 R. G. MARTIN, *J. Biol. Chem.*, 238 (1963) 257.
3 J. PATTE, G. LEBRAS, T. LOVING AND G. N. COHEN, *Biochim. Biophys. Acta*, 67 (1963) 16.
4 J. P. CHANGEUX, *Cold Spring Harbor Symp. Quant. Biol.*, 26 (1961) 313.
5 H. S. MOYED AND H. E. UMBARGER, *Physiol. Rev.*, 42 (1962) 444.
6 N. E. NEILSON, M. F. MACQUILLAN AND J. J. R. CAMPBELL, *Can. J. Microbiol.*, 5 (1959) 293.
7 E. R. STADTMAN, G. N. COHEN, G. LEBRAS AND H. DE ROBICHON-SZULMAJSTER, *J. Biol. Chem.*, 236 (1961) 2033.
8 D. P. STAHLY AND R. W. BERNLOHR, *Biochim. Biophys. Acta*, 146 (1967) 467.
9 H. PAULUS AND E. GRAY, *J. Biol. Chem.*, 242 (1967) 4980.

Received September 23rd, 1968