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ARGINASE INHIBITION

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Summary

L-Ornithine (K_i = 1.3 mM) and adenine (K_i = 0.7 mM) are competitive inhibitors and borate (K_i = 0.25 mM) is a noncompetitive inhibitor of arginase (L-arginine amidohydrolase, EC 3.5 3.1). In contrast to Rosenfeld et al. (Rosenfeld, J L., Dutta, S.P., Cheda, G B and Tritsch, G L. (1975) Biochim. Biophys. Acta 410, 165), we observe no inhibition of arginase by cytidine, cytosine or adenosine.

The purification of beef liver arginase (L-arginine amidohydrolase, EC 3.5.3.1) is difficult, requiring three column chromatography steps followed by preparative isoelectric focussing [1]. We are attempting to develop a more convenient purification utilizing affinity chromatography. Consequently, we were interested in the report by Rosenfeld et al [2] that several purines are competitive inhibitors of arginase and that cytosine and cytidine are noncompetitive inhibitors of arginase All of these compounds were reported to be potent inhibitors with K_i values ranging from 0 9 to 40 μM. In contrast, we report here that cytosine, cytidine and adenosine do not inhibit arginase and that adenine is a considerably less effective inhibitor than reported. In addition, K_i values are reported for the competitive inhibition of arginase by L-ornithine and for noncompetitive inhibition by borate.

Typical results from steady-state kinetic studies of beef liver arginase in the presence of various inhibitors are shown as Lineweaver-Burk plots in Fig 1. Rosenfeld et al [2] report that cytosine and cytidine are noncompetitive inhibitors of arginase with inhibition constants of 0.9 and 5 μM, respectively As

shown in Fig. 1, we observe no inhibition of arginase by 100 μM cytidine. Even at a concentration of 300 μM, cytidine, cytosine and adenosine show no significant inhibition of arginase. In agreement with Rosenfeld et al [2], we observe competitive inhibition of arginase by adenine but our data suggest a K_i of around 700 μM rather than 14 μM as reported by Rosenfeld et al. [2]. The velocities reported in Fig. 1 were determined by measuring urea formation using a coupled enzyme assay as described in Bergmeyer [3], while Rosenfeld et al [2] measured velocities using a direct spectrophotometric assay [5]. Using the same methods and concentration of cytidine used in their experiments (50 μM), we observed no inhibition of arginase

As a test of our methods, inhibition constants were determined for L-ornithine, a well-known competitive inhibitor [6], and borate, one of the most potent arginase inhibitors [7]. We find K_i = 1.3 for L-ornithine at pH 9.5. This is similar to a value of 3 ± 1 mM at pH 7.5 reported by Kuchel et al. [8] for purified beef liver arginase and to values of 1.3 mM [9] and 1.4 mM [10] for the arginase from rat and rabbit liver. Borate is clearly a noncompetitive inhibitor of arginase and the value of K_i is approx. 0.25 mM. The type of inhibition and K_i value for borate have not been previously reported.

The direct spectrophotometric assay used by Rosenfeld et al. [2] is based on

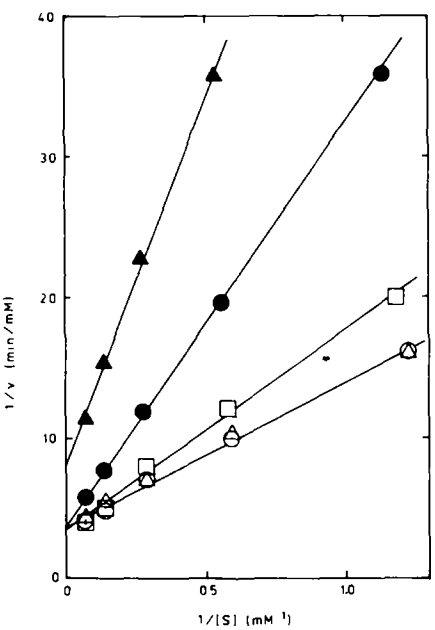

Fig 1 Inhibition of beef arginase by 1 mM borate (▲———▲), 2.4 mM L-ornithine (●———●), 0 3 mM adenine (□———□) or 0 1 mM cytidine (△———△) at pH 9 5, 37°C No inhibitor (○) Beef liver arginase (33 3 units/mg, Worthington Biochemical Corp) was dissolved in and dialyzed against 10 mM Tris-HCl/ 10 mM $MnCl_2$, pH 7 5 buffer 100 µl of the enzyme solution was added to 3 ml of an L-arginine solution (pH 9 5, 37°C) containing the inhibitor when indicated The reaction was terminated after 5 min and the amount of urea formed measured using the coupled enzyme assay described in Bergmeyer [3] Since the amount of substrate hydrolyzed approached 15% at the lowest substrate concentrations, corrections for substrate depletion were made as previously described [5] The K_i values reported are based on these experiments plus similar experiments at one other inhibitor concentration

the measurement of absorption changes at 206 nm. Consequently, they were able to use a single low inhibitor concentration (50 μM) and a very limited substrate concentration range (0.5—1.0 mM L-arginine). The disagreement with the results presented here probably results from these limitations. Our failure to observe inhibition at much higher inhibitor concentrations (300 μM) and our agreement with results in the literature for known inhibitors of arginase lend credence to our results. This research was supported by Grant AM 19 112 from the National Institute of Health.

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