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PURINE AND PYRIMIDINE INHIBITORS OF ARGINASE

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Summary

1. Adenosine, inosine, adenine and uric acid are competitive inhibitors, and cytidine and cytosine noncompetitive inhibitors of bovine liver arginase (L-arginine amidinohydrolase, EC 3.5.3.1).

2. The affinity of the enzyme for these inhibitors was 10—100 times as great as for substrate in terms of K_i versus K_m.

3. These nucleic acid metabolites may thus function in vivo to regulate the urea cycle.

4. Several naturally occurring competitive and noncompetitive inhibitors of arginase of unknown structure have been isolated from plant and animal tissue. From their properties and methods of isolation, they may be the purines and pyrimidines herein described.

5. These purines and pyrimidines have no effect on tryptic hydrolysis.

Introduction

There have been several reports of low molecular weight arginase inhibitors in animal tissues [1—3]. These inhibitors were not purified sufficiently to permit identification, but their properties and methods of isolation did not suggest them to be the products of the arginase reaction (i.e., ornithine, lysine or citrulline) which are known enzyme inhibitors [4—7]. Several other arginase inhibitors of known structure have been described [8—11] and these ostensibly resemble arginine by possessing a guanidine group. Reported herein are our findings that several naturally occurring purines are competitive inhibitors, and that pyrimidines are noncompetitive inhibitors of bovine liver arginase.

Experimental

Arginase (L-arginine amidinohydrolase EC 3.5.3.1) from bovine liver was obtained from Sigma Chemical Co. The assay for enzyme activity was based on

TABLE I

ARGINASE INHIBITORS

Compound	K_i (μM)	
Adenosine	30	Competitive
Inosine	40	
Adenine	14	
Uric acid	9	
Cytidine	5	Non-competitive
Cytosine	0.9	

the change in absorbance at 206 nm which accompanies the conversion of arginine into ornithine and urea [12]. Substrate concentrations between 0.5 and 1.0 mM adjusted to pH 9.4 were incubated at 37°C in a Cary model 14 spectrophotometer and the rate of change in absorbance recorded. Inhibitors were used at a concentration of 0.05 mM. Higher concentrations of substrate or inhibitors could not be used because of their absorbance at 206 nm. Double reciprocal plots of the data were constructed and linear regressions fitted by eye. Calculation of K_m and K_i was as described by Dixon and Webb [13].

Results

Table I summarizes our findings. Of the purines, the most potent competitive inhibitor was uric acid. If it may be considered that K_m for this enzyme approximates K_s, K_i may be compared to K_m to evaluate affinities of competitive inhibitors relative to substrate. On this basis, the compounds listed in the table are 10—15 times more tightly bound by the enzyme than substrate. Inosine appears to be the least tightly bound. For L-arginine, K_m was found to be 300—400 μM.

The pyrimidines had a higher affinity for the enzyme than the purines, but they were noncompetitive inhibitors. Cytosine had a higher affinity than the riboside.

Discussion

Several inhibitors of arginase are known. In addition to the urea cycle members [4—7], inhibitors of known structure include indospicine [9], chlorogenic acid and its derivatives [8], triphenyl methane dyes [14], DL-amino-l-guanidino-4-butyl phosphonic acid [11], and sulfonyl ureas [15]. Of particular interest are several reports of low molecular weight, water soluble, competitive and noncompetitive inhibitors which were stable at 100°C [1—3,8,16]. These were found in sunflowers and vertebrate and invertebrate tissues. These inhibitors were not sufficiently purified to permit identification, but their methods of purification and their properties in addition to their wide distribution suggest that they might be purines or pyrimidines. Thus, products of nucleic acid metabolism may influence the urea cycle in vivo. There is evidence for this type of control [17].

There are no guanidine groups in the inhibitors listed in Table I on which binding to arginase could be rationalized. However, it is possible that the N-C-N sequence within the heterocycles sufficiently resembles guanidine to account for the observations. Apparently, the purines more closely resemble arginine than the pyrimidines since the former were competitive inhibitors. In terms of K_i versus K_m, these compounds are comparable in affinity for enzyme to lysine and ornithine [7].

Because arginine is involved in directing the specificity of trypsin and thrombin and because some guanidine compounds have high affinity for the active site of trypsin [18], the purines and pyrimidines were also tested as inhibitors of the tryptic hydrolysis of *p*-toluenesulfonyl-L-arginine methyl ester [19]. No inhibitory activity was encountered for any of the compounds. Hence, there are clear differences in the portion of the arginine molecule which directs the specificities of these two enzymes.

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