Purine and Pyrimidine Nucleotide Inhibition of Carbamyl Phosphate Synthetase from Pea Seedlings

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End-product inhibition of glutamine-requiring carbamyl phosphate synthetase[1] derived from Escherichia coli and Saccharomyces cerevisiae has been studied by Lacroute, et al. (1965), Pierard, et al. (1965), Anderson and Meister (1966) and Pierard (1966). With E. coli carbamyl phosphate synthetase, UMP[2] was the most potent inhibitor tested, having a Ki of approximately 5 x 10^{-6} M (Anderson and Meister, 1966). Next in order of decreasing inhibition at 10^{-3}M, were UDP and UTP. Inhibition by these compounds, however, was sharply reduced by 6 x 10^{-3} M ornithine (Pierard, 1966). Studies with mutants have led to the conclusion that UMP and ornithine do not have a common binding site on the enzyme (Pierard, 1966). In contrast with pyrimidine nucleotides, several purine nucleotides significantly stimulate activity of carbamyl phosphate synthetase (Anderson and Meister, 1966).

[1] L-glutamine + $HOCO_2^-$ + 2 ATP^{-4} $\xrightarrow{Mg^{++},\ K^+}$ carbamyl phosphate[2] + 2 ADP^{3-} + $HOPO_3^{=}$ + glutamate

[2] Abbreviations: ATP=adenosine triphosphate, ADP=adenosine diphosphate, AMP=adenosine-5'-monophosphate, ITP=inosine triphosphate, UTP=uridine triphosphate, UDP=uridine diphosphate, UMP=uridine mophosphate, GDP=guanosine diphosphate, TMP=thymine-5'-monophosphate, CMP=cytosine-5'-monophosphate.

We have partially purified[1] the glutamine requiring enzyme catalyzing carbamyl phosphate synthesis in pea (Pisum sativum L. cv. Alaska) seedlings. It is subject to end-product inhibition by several pyrimidine nucleotide and some purine nucleotides. These findings will be discussed briefly here. More details will be provided elsewhere.

The enzyme preparations employed in these experiments were purified 10-13 fold from 10-15 day old pea seedlings. The enzyme was quite unstable, but was protected to some degree by 2-mercaptoethanol, L-glutamine, and L-ornithine.

RESULTS

A variety of compounds of possible regulatory significance were tested. From Table 1 it is apparent that several purine and pyrimidine nucleotides cause significant inhibition. Note that inhibition decreased markedly in the order UMP, TMP, and CMP. It is also of interest that ITP increased activity somewhat, whereas GTP and UTP were quite inhibitory.

Lineweaver-Burk plots were made to determine the inhibition by UMP, UDP, AMP, and ADP over a range of ATP concentrations. The results for UMP and ADP are shown in Figure I and II. Inhibition by UMP, UDP and AMP was competitive with ATP, while ADP was non-competitive with ATP. The approximate apparent Ki values for UMP, UDP, AMP, and ADP were, respectively, 2.0-4.0 x 10^{-6}M, 2.3 x 10^{-5}M,

[1]Up to 40-45 fold purification.

3.8×10^{-5}M, and 5.6×10^{-4}M. The Ki for UMP in tris-H_2SO_4 buffer was 1.9-2.0 $\times 10^{-6}$M, while in tricine-KOH buffer the Ki was 4×10^{-6}M. Curvilinear double reciprocal plots of the activity of the enzyme in the presence of UMP, were obtained, as shown in Figure II. Hyperbolic kinetics were expecially noticeable for UMP inhibition using tris-H_2SO_4 buffer, and this may indicate an allosteric alteration in the enzyme.

Finally, it was found that increasing concentrations of L-ornithine-HCl significantly decreased inhibition by UMP. Different lots of carbamyl phosphate synthetase were used to test this point. In one study ornithine-HCl decreased inhibition by 3×10^{-5}M UMP from 80% at 0.2 mM ornithine-HCl to only 30.8% at 3.5 mM ornithine-HCl. In another study the inhibition was 69.3% and 13.3% with respective ornithine-HCl concentrations of 0.2 mM and 4mM. Still other studies gave varying values; sometimes significantly less reduction of UMP inhibition by ornithine-Hcl occurred than in the above examples. In two experiments, double reciprocal plots indicated that ornithine-HCl competitively decreased UMP inhibition, whereas in two other experiments the decreased inhibition in the presence of ornithine-HCl was more complex kinetically.

Ornithine-HCl was much more effective in reducing inhibition by UMP than in reducing inhibition by UDP, AMP and ADP.

Table 1

Compounds Tested as Inhibitors of Pea Carbamyl Phosphate Synthetase[a]

	Compound	Concentration mM	Relative Activity Per cent
	Control	--	100
	AMP	6	18
	ADP	6	1.6
Experiment I[b]	ureidosuccinate	6	93.5
	orotic acid	2	100.7
	L-arginine	6	95
	GTP	4	31.3
	UTP	4	37.6
	ITP	2.5	115.3
	fumarate	2	85
	Control	--	100
	UMP	5	13
	UDP	5	11.5
Experiment II[c]	TMP	5	36
	CMP	4	70
	GDP	3	86
	canavanine	3	73
	glutamate[d]	5	78

[a]Incorporation of $^{14}CO_2$ into acid-stable ^{14}C-citrulline was the basis of the assay procedure. Assays were performed at 37°C for 8-10 minutes, in tubes containing ATP, Mg^{++}, L-ornithine, L-glutamine, 1.4 mM 2-mercaptoethanol, 10 units ornithine transcarbamylase, and 0.1 mM $NaH^{14}CO_3$ (specific activity of 2.1×10^6 cpm/mM), in a total volume of 0.25 ml. The reaction was ended by pipetting 0.2 ml. of the assay mixture into 0.25 ml. of ethanol -2.5 N HCl (2:1, v/v) plus a drop of dilute detergent on a 2.5 cm. stainless steel planchet. After evaporating to dryness under a 150-watt incandescent lamp, the readioactivity was measured with a Vaird-Atomic Model 135 scaler.

[b]Assay conditions Expt. I: ATP = 3 mM, Mg^{++} = 13 mM, ornithine = 1.2 mM, glutamine = 3.0 mM, enzyme = 0.04 mg, tricine-KOH buffer pH 8.1 = 0.05 M.

[c]Assay conditions Expt. II: ATP = 1.2 mM, Mg^{++} = 10 mM, ornithine = 0.8 mM, glutamine - 1.0 mM, enzyme = 0.045 mg, tricine-KOH buffer pH 8.1 - 0.05 M.

[d]L-glutamine = 4.0 mM.

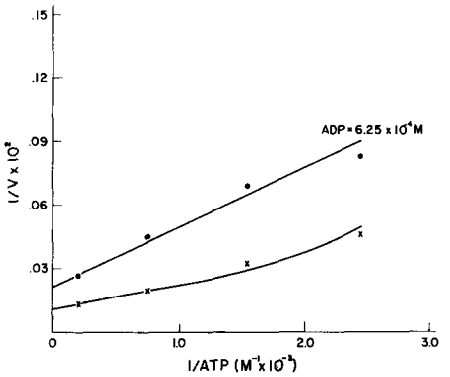

Figure I

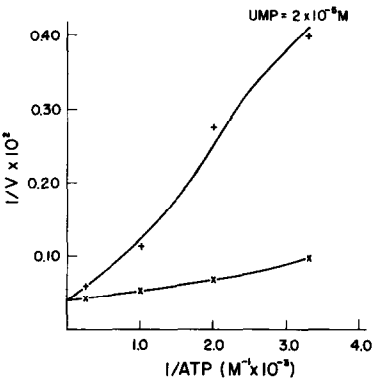

Figure II

Figure I. Inhibition of Alaska pea carbamyl phosphate synthetase by ADP. Reaction run at 37°C for 10 min in 0.05 M pH 8.2 tris-H_2SO_4 buffer. $MgSO_4$ = 14 mM, 2-mercaptoethanol = 1.4 mM, ornithine HCl = 1.3 mM, L-glutamine = 1.5 mM, K_2SO_4 = 5mM, ornithine transcarbamylase = 20 units, $NaH^{14}CO_3$ = 0.1 mM. -x-x-x = minus ADP; -o-o-o = plus 6.25 x 10^{-4} M ADP.

Figure II. Inhibition of Alaska pea carbamyl phosphate synthetase by UMP. Reaction run for 10 min in pH 8.2 tris-H_2SO_4 buffer. $MgSO_4$ = 12.5 mM, ornithine-HCl = 1.0 mM, L-glutamine = 1.25 mM. Concentration of other reagents as in Figure I. -x-x-x = minus UMP; -+-+-+ = plus 2 x 10^{-5} M UMP.

SUMMARY

Carbamyl phosphate synthetase from Alaska pea seedlings is subject to inhibition by several pyrimidine and purine nuclotides. It is similar in some respects to the E. coli enzyme. Both have similar Ki values for UMP, the most potent inhibitor, and both show partial reversal of UMP inhibition by L-ornithine. However, the pea enzyme is inhibited by AMP and ADP, while the E. coli enzyme is stimulated by these compounds.

The role of nucleotides and ornithine in strongly influencing the activity of carbamyl phosphate synthetase has obvious potential regulatory significance in the operation of the ornithine cycle and in pyrimidine nucleotide synthesis.

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