and chromosomal polymorphism appear to combine in an interlocking system to ensure heterozygosity. Perhaps this system permits the species to enjoy hybrid vigor while allowing it to avoid the penalty of large numbers of inviable gametes or zygotes.

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Control of the Activity of Escherichia coli Carbamoyl Phosphate Synthetase by Antagonistic Allosteric Effectors

Abstract. *The synthesis of carbamoyl phosphate required in both arginine and pyrimidine biosyntheses is carried out by a single enzyme in* Escherichia coli. *Opposed effects of pyrimidine nucleotides and of ornithine on the activity of the enzyme ensure a proper supply of carbamoyl phosphate according to the needs of the two biosynthetic sequences.*

Several regulatory patterns for single enzymes supplying divergent metabolic pathways with a common precursor have been encountered so far. For instance, control mechanisms such as multivalent repression (*1*), concerted feedback inhibition (*2*), cumulative feedback inhibition (*3*), or specific reversal of feedback inhibition (*4*) provide effective means of avoiding the regulatory interactions which otherwise could arise from such situations.

The regulation of the enzymic system which supplies carbamoyl phosphate (CP) for the synthesis of arginine and the pyrimidines in *Escherichia coli* is the subject of my report. Although this double function of CP has been known (*5*), definitive knowledge of the mechanism of its formation came only recently with the discovery, first in mushrooms (*6*) and later in *E. coli* (*7*), of an enzyme, glutamino-carbamoyl phosphate synthetase, which uses glutamine as the carbamoyl nitrogen donor (*8*). There is evidence that in *E. coli* a single glutamino-carbamoyl phosphate synthetase provides CP for both the arginine and pyrimidine pathways (*9*). The synthesis of the aforesaid enzyme is cumulatively repressed by the end products of the two pathways while its activity is subject to feedback inhibition by a pyrimidine nucleotide, uridine-5′-monophosphate (UMP).

Under the conditions used, the inhibition was no greater than 60 percent, even for UMP concentrations exceeding $10^{-2}M$. This partial feedback inhibition by UMP was considered essential in order to allow for the possibility of producing carbamoyl phosphate necessary for the biosynthesis of arginine. Similar cases of partial inhibition have been observed in the control of homoserine dehydrogenase from *E. coli* and *Rhodospirillum rubrum* (*10*). I now present a more complete picture of the control of the activity of glutamino-carbamoyl phosphate synthetase in *E. coli* based on a heretofore overlooked involvement of ornithine in that control.

Glutamino-carbamoyl phosphate synthetase was previously assayed by an indirect method based on the coupling of the synthesis of CP with ornithine-carbamoyl phosphate transferase (OCT), the citrulline formed being estimated colorimetrically (*7*). However, a direct method was used in my work. It involves accumulation of CP during a main incubation, followed by its conversion into citrulline by a short additional incubation in the presence of an excess of ornithine and OCT (legend to Fig. 1). This method avoids the constant presence of ornithine during CP synthesis, and its use enables one to study the influence of this amino acid on the activity of the synthetase. The direct assay, if one takes into account the chemical decomposition of CP and a slight activation of the enzyme by ornithine, gives the same results as the coupled assay.

Under these conditions, the enzyme appears much more sensitive to UMP inhibition, which is close to 100 percent for $2.5 \times 10^{-3}M$ UMP (Fig. 1). Ornithine, while slightly increasing the activity when taken individually, reduces considerably the efficiency of UMP as an inhibitor of the enzyme (Fig. 1). This effect of ornithine is obtained under conditions where it does not participate in the removal of CP through coupling with OCT. Ornithine may thus be seen as an allosteric effector of glutamino-carbamoyl phosphate synthetase which is responsible for the previously observed limitation of the feedback inhibition by UMP.

The study of the specificity of the feedback inhibition has shown that, although UMP is the most potent negative effector of the aforesaid synthetase, other pyrimidine nucleotides share this property. In decreasing order of effectiveness, uridine diphosphate, uridine triphosphate, cytidine monophosphate, cytidine triphosphate, and cytidine diphosphate are inhibitors of the enzyme but all are antagonized by ornithine in this effect (Table 1). The activity of the synthetase is thus un-

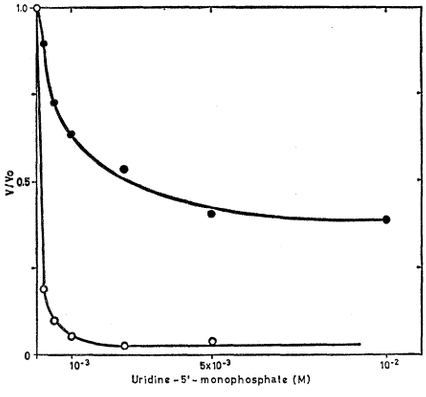

Fig. 1. Influence of ornithine on the feedback inhibition of *E. coli* glutamino-carbamoyl phosphate synthetase by UMP. This enzyme was extracted and partially purified (*8*), from the strain P4X of *E. coli* K12. The cells were grown on minimal medium No. 132 (*16*). The reaction mixture for the assay of the enzyme contained: $KHCO_3$, 30 μmole; ATP, 12 μmole; $MgCl_2$, 12 μmole; glutamine, 12 μmole; phosphate buffer (*p*H 7.5), 100 μmole; and enzyme in a total volume of 1 ml. This mixture was incubated for 15 minutes at 37°C. At this point, 1500 units of partially purified OCT from *E. coli* and 6 μmole of ornithine were added. The incubation was continued for 2 minutes in order to convert CP into citrulline. The reaction was stopped, and citrulline was determined (*7*). The activities obtained were corrected for the amount of CP formed during the additional 2-minute incubation period in the presence of ornithine and OCT. Solid circles, the reaction mixture contained ornithine from the start of the incubation; OCT was added after a 15-minute incubation period. Open circles, ornithine and OCT were added after 15 minutes incubation.

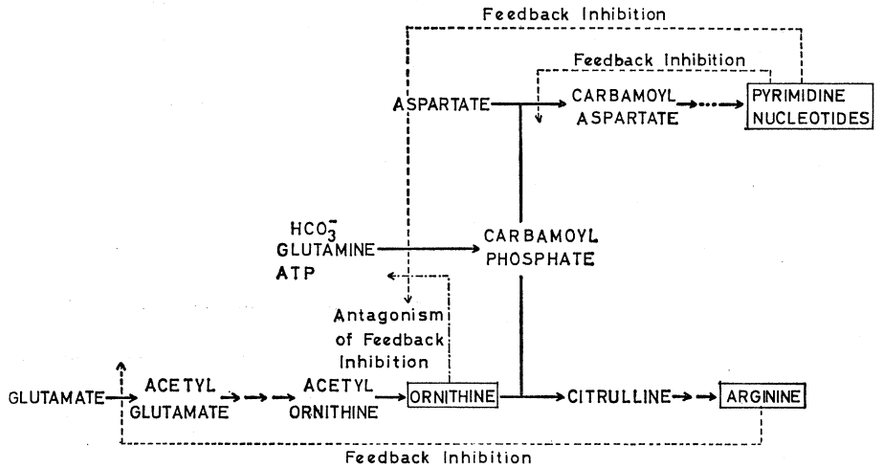

Fig. 2. Control of the activities in the arginine and pyrimidine biosynthetic pathways of *E. coli*.

Table 1. Specificity of the feedback inhibition of *E. coli* glutamino-carbamoyl phosphate synthetase.

Concentration of the inhibitor	Activity* in presence of							
	Uridine	UMP	UDP	UTP	Cytidine	CMP	CDP	CTP
	No ornithine							
$5 \times 10^{-4}M$	96	12	31	83	99	102	101	89
$5 \times 10^{-3}M$	95	4	10	24	102	38	84	57
	6×10^{-3}M ornithine							
$5 \times 10^{-4}M$	102	90	99	100	100	98	102	103
$5 \times 10^{-3}M$	100	52	74	91	98	102	105	92

* Activities are expressed as percent of the activity without inhibitor. The enzyme assay is performed as described in legend of Fig. 1.

der control of the general pool of pyrimidine nucleotides in the cell. In contrast with the rather low specificity of the inhibitor, ornithine is highly specific in counteracting that inhibition. Several intermediates of the arginine pathway, including acetylglutamate [an activator of the carbamoyl phosphate synthetase (*11*) active in the urea cycle], acetylornithine, citrulline, and arginine, did not significantly affect the activity of the enzyme, whether tested alone or in the presence of UMP. Aspartate, the symmetric counterpart of ornithine in the pyrimidine pathway, did not influence the activity or decrease the inhibition by UMP.

Although it is not possible to propose a detailed molecular model of glutamino-carbamoyl phosphate synthetase according to the current views on allosteric proteins (*12*), as this will require a thorough kinetic study of the enzyme, an interesting feature has already been established. Indeed, from the fact that a hyper-retro-inhibited mutant (*9*) has lost the antagonism by ornithine of the feedback inhibition, it may be inferred that ornithine and UMP act on different sites of the enzyme.

The advantages of the antagonistic effects of the pyrimidine nucleotides and of ornithine on the control of glutamino-carbamoyl phosphate synthetase are obvious; a scheme of the control of the enzymic activities in the two biosynthetic pathways which are tributary of CP is presented in Fig. 2. An increase of the intracellular concentration of pyrimidine nucleotides affects the activity of glutamino-carbamoyl phosphate synthetase and of aspartate carbamoyltransferase (*13*), and thus the rates of synthesis of CP and pyrimidines are reduced. The inhibition of the synthetase remains maximum as long as no need for arginine synthesis occurs. Indeed, a high intracellular concentration of arginine leads to feedback inhibition of acetylglutamate synthetase (*14*), the first enzyme of the arginine sequence. Therefore, the flow of metabolites in direction of ornithine is stopped and the carbamoyl phosphate synthetase remains under the direct control of the pool of pyrimidine nucleotides. A limitation of the arginine concentration, by relieving the inhibition of acetylglutamate synthetase, will open the door to the flow of metabolites through the arginine pathway and lead to some ornithine accumulation. Ornithine will reverse the inhibition by UMP (ornithine concentrations no greater than $5 \times 10^{-4}M$ cause a significant decrease of UMP inhibition) and ensure the supply of the arginine pathway with CP according to its needs.

Finally, the mode of control of a single glutamino-carbamoyl phosphate synthetase, which is described here, allows regulation of CP synthesis that is as efficient as that in *Saccharomyces cerevisiae* with two independently regulated enzymes (*15*).

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