REGULATION AND MUTATION AFFECTING A GLUTAMINE DEPENDENT FORMATION OF CARBAMYL PHOSPHATE IN ESCHERICHIA COLI

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Carbamyl phosphate (CP) is the common donor of the carbamyl group for the biosynthesis of arginine and the pyrimidines. In this line, the enzymic studies (Jones et al., 1956; Cohen, 1962) are in agreement with the occurrence in bacteria of single mutations resulting in a double auxotrophy for arginine and uracil (Roepke et al., 1944; Davis, 1962).

Three types of reactions yielding CP have been described :

1.- The irreversible formation of CP from CO_2, NH_4^+ and 2 ATP catalyzed by carbamyl phosphate synthetase. The enzyme requires Mg^{++} and an "activator" such as acetyl-glutamate (Cohen, 1962).

2.- The reversible phosphorylation by ATP of carbamate resulting from the chemical condensation of CO_2 and NH_3. This reaction, catalyzed by carbamyl phosphokinase (CPKinase), has been found in many microorganisms where it is generally considered as responsible for the synthesis of CP (Jones et al., 1956). As recently pointed out (Beckwith et al., 1962; Thorne and Jones, 1963), this opinion cannot be accepted without reservation. To satisfy the thermodynamic and kinetic needs of the forward reaction, the substrates concentrations and the pH have to be almost unphysiological. Moreover, contrarily to what should be expected, there is little correlation between the double auxotrophy for arginine and uracil and the absence of CPKinase (Kanazir et al., 1959; Schwartz, 1959; Beckwith et al., 1962; Thorne and Jones, 1963).

3.- A third reaction has been recently discovered in Agaricus bisporus (Levenberg, 1962). In this reaction, the carbamyl nitrogen donor is glutamine; CO_2, ATP and Mg^{++} are required and the optimal pH is 7.5. No reversibility is observed. This reaction does not raise the kinetic and thermodynamic difficulties of the reaction catalyzed by CPKinase.

We present data in favour of the role of the third reaction as the system supplying CP for the biosynthesis of arginine and the pyrimidines in E. coli :

The occurrence of Levenberg's reaction in E. coli (table I).
Cell free extracts of E. coli K_{12} strain P4X catalyze the synthesis of citrulline in the presence of glutamine, bicarbonate, ATP, Mg^{++} and ornithine. Maximum activity can be reached only after treatment of the extract on Sephadex, suggesting the presence of an inhibitor in the crude extract. Ornithine is essential for the ultimate transformation of the carbamyl group into citrulline. A concentration of NH_4^+ equivalent to that of glutamine shows a hardly significant activity.

The K_m for glutamine in the coli system is 4.10^{-4}M, a value which is close to that of the mushroom system. This K_m fits better the normal physiological conditions than the high K_m (.05 to .2 M) measured for carbamate with CPKinase (Beckwith et al., 1962; Thorne and Jones, 1963).

E. coli P4X grows on a rich medium free of arginine and uracil (AUF medium) with a generation time of 40 min. We calculate from this that the specific activity for CP synthesis must be about 1 unit. A specific activity for the glutamine dependent reaction of 0.8 unit has been measured. This value is thus roughly sufficient to account for growth at the observed rate. This was to be expected since Novick and Maas (1961) have shown that, during the growth of E. coli on a rich arginine free medium (AF medium), CP formation is rate-limiting.

Preliminary observations in this laboratory have shown the presence of the glutamine dependent reaction in Saccharomyces cerevisiae. Ory et al. (1954) found indirect evidence for a participation of glutamine in arginine synthesis

in Lactobacillus arabinosus. Such results suggest that the reaction could be widely distributed among microorganisms.

Table I

REQUIREMENTS FOR CARBAMYL PHOSPHATE SYNTHESIS FROM GLUTAMINE BY AN EXTRACT OF E. COLI P4X GROWN IN AUF MEDIUM°

N°	Incubated mixture	Activities (μMoles citrulline mg proteins^{-1} hour^{-1})
1	Complete	0.72
2	n° 1 without glutamine	0.02
3	n° 1 without $KHCO_3$	0.17
4	n° 1 without ATP	0.04
5	n° 1 without Mg^{++}	.0
6	n° 1 without ornithine	.0
7	n° 1 without extract	0.01
8	n° 2 plus 12 μMoles NH_4Cl	0.03

° Cells are grown aerobically at 36°C in an AUF (n° 421) medium. This medium is similar to the AF medium of Novick and Maas (1961), but uracil and cytosine are omitted. Growth in this medium, which contains vitamins, nucleic bases and amino acids, reduces the cellular pool of arginine and the pyrimidines and results in a derepression of the corresponding biosynthetic pathways.

Cells are collected at the end of the exponential growth phase. Cells, corresponding to about 100 mg of proteins, are suspended in 10 ml of 0.05 M phosphate (K) buffer, pH 7.5 and sonically disrupted for 5 min. (Raytheon 10 Kc). After centrifugation at 10,000 x g for 15 min., the supernatant is passed through a column of Sephadex G 25.

Carbamyl phosphate synthesis is assayed by coupling the reaction with ornithine transcarbamylase (OTC). The complete reaction mixture contains (in μMoles) for 2 ml of total volume : phosphate (K) pH 7.5, 175; glutamine, 12; $KHCO_3$, 60; ATP, 24; $MgCl_2$, 24; ornithine, 12; an excess of partially purified OTC from E. coli and extract. This mixture is incubated at 37°C for 30 min. The reaction is stopped by the addition of 2 ml of 2 N HCl followed by 10 min. boiling. The precipitated proteins are removed and aliquots are assayed for citrulline by a modification of the method of Archibald (1944). Values given are corrected for controls where the reaction is stopped at zero time.

The regulation of the glutamine dependent CP synthesis.

Much work has been devoted to the study of the metabolic control of CPKinase, assuming a role as a common step for the biosynthesis of arginine and uracil. The results are complex and difficult to sum up (Schwartz, 1959; Beckwith et al., 1962; Thorne and Jones, 1963; Gorini and Kalman, 1963).

In our hands (table II), the level of CPKinase in the strain P4X, prototroph for arginine and uracil, is not markedly influenced by the addition of arginine and uracil to the AUF medium.

In contrast to this, a ten fold decrease of the glutamine dependent reaction is observed in the same conditions. If the AUF medium is supplemented with either arginine or uracil alone, this activity is reduced to about 50 % of the original level.

The lack of the glutamine reaction in one-step mutants simultaneously auxotroph for arginine and uracil.

Four different strains have been used in this part of the work. Strain P678 is prototroph for arginine and uracil. Its derivative, P678B1, is a one-step mutant simultaneously auxotroph for arginine and uracil. In another mutant derived from P678, PA1066, a double auxotrophy has been obtained by two separate mutations (acetyl-glutamate synthetase is missing in the arginine pathway and a second block exists in the pyrimidine sequence between ureidosuccinate and uridine-5'-phosphate). RC50, a mutant from an unrelated strain, is similar to P678B1.

As shown in table II, the level of CPKinase is not altered in the double auxotrophs (P678B1 and RC50), although some derepression of this enzyme occurs after growth on limiting amounts of uracil.

In contrast to P4X, the glutamine dependent activity in all the mutants examined here is repressed to a barely detectable level by addition of arginine and uracil. However, the growth of PA1066 in chemostat on limiting amounts of either arginine or uracil leads to a significant derepression of the glutamine dependent synthesis of CP. No such derepression can be obser-

Table II

CARBAMYL PHOSPHOKINASE AND GLUTAMINE DEPENDENT CARBAMYL PHOSPHATE SYNTHETIZING ACTIVITIES AFTER DIFFERENT GROWTH CONDITIONS

Strain of *E. coli* K_{12}	Additions to the AUF medium ($\mu g, ml^{-1}$)	Type of culture	Activities (μMoles citrulline $mg\ proteins^{-1}\ hour^{-1}$)			
			CPKinase		Glutamine dependent CP synthesis	
			Exp 1	Exp 2	Exp 1	Exp 2
P4X	none	Batch	6.6	7.1	0.92	0.74
	Arginine 200	Batch	9.2	8.4	0.54	0.53
	Uracil 100	Batch	8.5	9.2	0.50	0.44
	Arginine 200, Uracil 100	Batch	4.6	6.8	0.05	0.08
P678	Arginine 200, Uracil 100	Batch	3.8	-	< 0.02	-
P678B1	Arginine 15, Uracil 100	Chemostat	3.4	-	< 0.02	-
	Arginine 200, Uracil 8	Chemostat	9.2	-	< 0.02	-
PA1066	Arginine 15, Uracil 100	Chemostat	3.8	-	0.19	-
	Arginine 200, Uracil 5	Chemostat	7.9	-	1.85	-
RC50	Arginine 200, Uracil 100	Batch	4.6	-	< 0.02	-
	Arginine 10, Uracil 100	Chemostat	3.7	-	< 0.02	-

See table I for growth conditions and assay of the glutamine dependent activity. CPKinase is assayed by the method of Jones et al. (1956), adapted to *E. coli* by Schwartz (1959). When available, the data obtained in two independent experiments are presented. Where indicated, growth was performed in a chemostat with a doubling time of 4 hours.

ved for P678B1, or RC50 after growth in the same conditions, a proof that these mutants are lacking the glutamine dependent reaction.

The data presented in this paper support the opinion that glutamine is the functional donor of carbamyl group for the arginine and pyrimidines biosynthetic pathways. The mechanism of the reaction(s) and of its genetic determinants deserve more extensive work. Particularly the cumulative effect of the two corepressors is an interesting case of regulation.

Strains RC50 and PA1066 have been obtained from R. Lavallé.

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