THE EFFECT OF GLYCINE ON THE URACIL INHIBITION OF ASPARTATE TRANSCARBAMYLASE SYNTHESIS IN ESCHERICHIA COLI W

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Proline or, more effectively proline peptides, increases 2-fold the amount of uracil necessary to inhibit the synthesis of aspartate transcarbamylase in Streptococcus lactis 8039 (Ravel, Hunter, and Shive 1959). The addition of the peptides of amino acids other than proline to an amino acid medium (Ravel, Woods, Felsing, and Shive 1954) has no appreciable effect upon the uracil inhibition of enzyme synthesis. In studying the uracil inhibition of aspartate transcarbamylase synthesis in Escherichia coli W, glycylproline and prolylglycine were found to overcome the uracil inhibition to the same degree as that demonstrated in S. lactis. Further investigation, however, revealed that this effect in E. coli W is apparently caused by the glycine moiety since peptides of glycine not containing proline(and, at higher concentrations, glycine itself)are effective. In contrast, proline and other amino acids and a number of peptides not containing glycine are ineffective.

Cells of E. coli W were harvested after 12 hours growth at 37° in a salts-glucose medium (Davis and Mingioli 1950) modified to contain 0.5% lactate and supplemented with 0.15 μmoles per ml. of uracil. The cells were washed and resuspended in fresh medium supplemented as indicated below. After 2 hours incubation at 37°, the cells were again harvested, suspended in 0.04 M Tris buffer, pH 8.5, and exposed to sonic oscillation in a Raytheon 10 kc.

oscillator for 5 minutes. Proteins were determined (Lowry et al. 1951), and the cell extracts were assayed for aspartate transcarbamylase by incubating an appropriate rate-limiting amount of extract with carbamyl phosphate, 10 μmoles; L-aspartate, 20 μmoles; and Tris buffer, 20 μmoles, pH 8.5, in a total volume of 1 ml. After 15 minutes at 25°, the reaction was stopped by passage through a 1 ml. Dowex 50 (H^+ form) column. The column was washed with 2 ml. of water and the effluent assayed for carbamylaspartate (Koritz and Cohen 1954).

The sonic extract of cells harvested after 12 hours growth in the presence of uracil had a specific activity of 2.5 (μmoles of carbamylaspartate formed per mg. of protein per hour). If the cells were allowed to grow for 2 hours in the absence of an exogenous supply of uracil, the sonic extract of these cells had a specific activity of 36. As seen in Table I, an exogenous

Table I

Reversal of Uracil Inhibition of Aspartate Transcarbamylase Synthesis in E. coli W

Supplement[a]	μmoles/ml.	Per cent of Control[b]
None		20
"	0.1	87
Glutathione	0.04	106
Glycylglycine	0.04	110
L-Alanyl-L-phenylalanine	0.04	17
Glycyl-L-phenylalanine	0.04	105
L-Histidyl-L-histidine	0.04	10
Glycine	0.4	105
L-Glutamic Acid	2.0	18
L-Proline	2.0	22
Glycyl-L-proline	0.04	55

[a] All contained 0.04 μmoles/ml. of uracil.
[b] The control was a sonic extract of cells which had been incubated for 2 hours in the absence of uracil and had a specific activity of 36.

supply of uracil (0.04 μmoles per ml.) prevents the synthesis of aspartate transcarbamylase. A number of glycine peptides were tested and all are effective in overcoming the inhibitory action of this concentration of uracil. Glycine, at 10 times the concentration of the glycine peptides, similarly increases 2-fold the amount of uracil necessary to prevent enzyme synthesis. Peptides which do not contain a glycine moiety are inactive, and of the 18 amino acids tested, only glycine and, less effectively, threonine, which is known to be converted to glycine, are active.

It appears that the site of inhibition of enzyme synthesis by uracil, or more probably a derivative of uracil, may occur at an advanced stage such that derivatives of particular amino acids may become limiting. The ability of amino acids to affect uracil inhibition suggests that the inhibition may involve nucleotide-amino acid derivatives.

Preliminary investigations with a glycine deficiency in S. lactis indicate that there is an interdependence of proline and glycine in reversing the uracil inhibition. Further studies with these and other microorganisms are in progress.

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