CYSTEINE INHIBITION OF THE RELEASE OF REPRESSION OF DIHYDROOROTIC ACID DEHYDROGENASE IN ESCHERICHIA COLI

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During studies on the biosynthesis of dihydroorotic acid (DHO) dehydrogenase in cells of E. coli, it was observed that a complete mixture of amino acids failed to substitute for $(NH_4)_2SO_4$ as a nitrogen source. Further examination of this phenomenon showed that the presence of cysteine prevents the usual burst of DHO dehydrogenase synthesis that normally occurs when the repressor, uracil, is removed. Of a large series of sulfur-containing compounds tested, substantial inhibition of the release of repression was observed with cysteine, cystine, L-cysteine ethyl ester, S-methyl cysteine, 2,3-dimercaptopropanol, 2,4-dithiopyrimidine, and β-mercaptoethanol, but not with methionine, homocystine, glutathione, taurine, thioglycollic acid, and cystamine.

Cells of E. coli K 12-496 (uracilless mutant) were grown in a minimal medium with glucose and uracil at 37°. After 17 hr incubation the culture was diluted 5-fold and further incubated for 3 hr to obtain log phase cells. The cells were harvested, washed once with 0.01 M Tris buffer pH 7.5 and resuspended in minimal medium with or without additives. At suitable time intervals 5 ml of culture was removed, cooled, and treated with 0.25 ml of toluene at 37° for 20 min. The toluenized cells were removed by centrifugation and resuspended in 5.0 ml of distilled water. DHO dehydrogenase activity was measured as described by Yates and Pardee (1957). Toluenized cells gave

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the same activity as sonic extracts or extracts prepared with a French cell. Cysteine does not interfere in the assay of DHO dehydrogenase. DHO dehydrogenase activity is expressed as specific activity, i.e., μmoles of orotic acid formed per hr per mg protein. Some typical results are shown in Table I where the data are presented in percent increase in specific activity of DHO dehydrogenase in 30 min. In the absence of uracil this value is about a 10-fold increase, i.e., from 0.39 to 4.36 in 30 min.

Table I

Effect of Uracil and Various Sulfur-Containing Compounds on the Release of Repression of DHO Dehydrogenase

Additions	Molar concn.	% Increase in DHO dehydrogenase in 30 min
None		100
Uracil	4.5×10^{-5}	30.3
Uracil	9.0×10^{-5}	4.7
Cysteine	8.25×10^{-4}	6.0
Cystine	"	6.0
S-Methyl cysteine	"	15.0
L-Cysteine ethylester	"	20.0
2,3-Dimercaptopropanol	"	18.0
2,4-Dithiopyrimidine	1.8×10^{-4}	17.0
β-Mercaptoethanol	8.25×10^{-4}	53.0
Methionine	"	97.0
Homocystine	"	93.0
Glutathione	"	103.0
Thioglycollic acid	"	88.0
Taurine	"	83.0
Cystamine	"	79.0

From the data of Table I no specific structure seems to be required for the inhibitory effect. However, certain generalizations are suggested. All the inhibitory compounds contain sulfur and it does not seem to be essential that the sulfur be -SH,

since -S-S- and CH_3-S are also effective. If one contrasts S-methyl cysteine and cystine with their respective homologues, methione and homocystine, it would seem that the grouping X-C-C-S is effective for inhibition, whereas X-C-C-C-S is not. When X is NH_2, the compound is more effective than when X is OH--compare cysteine with mercaptoethanol. Also when NH_2 is substituted, as in glutathione, the inhibitory character is lost. The carboxyl group also seems to have a potentiating effect since cystamine, the decarboxylated analogue of cystine, is inactive. These generalizations break down when disulfide compounds are considered. Although no specific generalizations can be made with the present data, there is a suggestion that the inhibitory compounds might be metabolized to a common specific structure that is the actual inhibitor.

Unlike the case of aspartyl transcarbamylase (Ravel, Hunter, and Shive, 1959), where glycine can release uracil repression, neither glycine nor proline is able to release the cysteine inhibition. Somewhat similar to the behavior of aspartyl transcarbamylase, the uracil repression of DHO dehydrogenase can be overcome about 50% by glycine, but this seems to be the maximum release that can be obtained regardless of the concentration of glycine used. The effect of cysteine on DHO dehydrogenase seems to be specific, since cysteine does not have a similar effect on the release of aspartyl transcarbamylase or ornithine transcarbamylase in the same organism (Taylor and Novelli, 1959). That the cysteine inhibition of DHO dehydrogenase synthesis is not identical with uracil repression can be inferred from the fact that the effects of the two compounds are not additive. Indeed, uracil in low concentration (1-2 μg) is capable of reversing the cysteine inhibition. Cysteine, although not obviously on the biosynthetic pathway to pyrimidine synthesis, can nevertheless mimic the action of the repressor uracil at least for DHO dehydrogenase. This finding, together with the report of Ravel, Hunter, and Shive, further implicates amino acids in the repression and release of repression of pyrimidine biosynthesis in E. coli. A detailed account of this investigation will be published elsewhere.

References

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