THE PREFERENTIAL INHIBITION BY CHLORAMPHENICOL OF INDUCED ENZYME SYNTHESIS

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The inhibition of protein synthesis by chloramphenicol and other antibiotics is well known. Chloramphenicol inhibits the transfer of amino acids from s-RNA to ribosomal protein (Nathans and Lipmann, 1961), but not the activation of amino acids (DeMoss and Novelli, 1956) or the transfer of activated amino acids to soluble RNA (Lacks and Gros, 1956). The synthesis of all proteins can be inhibited by sufficiently high concentrations of chloramphenicol (Aronson and Spiegelman, 1962). During a study with sub-lethal concentrations of chloramphenicol in this laboratory, it was noted that the specific activities of two inducible enzymes in Escherichia coli, β-galactosidase and D-serine deaminase, were lower in chloramphenicol treated cultures than in control cultures (Nakaya and Treffers, 1959). This decrease in specific activity for these enzymes indicated a preferential inhibition of their synthesis. This phenomenon of preferential inhibition is being studied further, and is the subject of this preliminary report.

Methods. The K-12 strain of Escherichia coli was used throughout. The bacteria were grown in mineral salts medium (Rickenberg, et al., 1953) with 0.5% glycerol as carbon and energy source. β-galactosidase was induced with thiomethyl-β-D-galactoside (TMG) at a final concentration of 10^{-3} M and was assayed by the method described by Rickenberg, et al. (1953) using toluenized cells. The induction and assay of tryptophanase and citritase were carried out as described by Yanofsky (1955), and Dagley and Dawes (1955) respectively. The methods of Pardee and Prestidge (1955) were employed for D-serine deaminase induction and assay, except that 0.05% yeast extract was

added to the assay buffer. Acid phosphatase activity was measured by the method of Torriani (1960). Hexokinase was assayed by the spectrophotometric determination of acid liberation, and glucose-6-phosphate dehydrogenase activity by the reduction of TPN at 340 mμ. The accumulation of radioactivity from lactose-C^{14} was used as a measure of galactoside permease activity in a β-galactosidase negative, permease positive mutant. Radioactivity accumulation was determined by the membrane filter technique (Horecker, et al.,1960). Inorganic phosphate liberation was used to measure pyrophosphatase activity.

Results and Discussion. The kinetics of β-galactosidase induction in chloramphenicol-treated cells were studied. In all cases the antibiotic was employed at concentrations below 1.0μg/ml, which permitted exponential growth at 50-60% the control rate. Fig. 1 is a differential plot of enzyme vs. protein, and shows that β-galactosidase synthesis is inhibited to a greater degree than total protein synthesis. This same degree of inhibition was obtained when the antibiotic was added either 60 min before or 60 min after the addition of inducer. In control experiments, the activity of the enzyme was unaffected by the antibiotic. Experiments using extracts prepared in the French Press or by sonication yielded the same results as those obtained with toluenized cells.

We examined the possibility that the preferential inhibition of β-galactosidase synthesis was due to competition by chloramphenicol (CAP) for phenylalanine or other aromatic amino aicds (cf. Woolley, 1950), formation of enzymatically inactive, cross-reacting protein, or degradation of the enzyme during chloramphenicol treatment. Bacteria were pre-incubated with DL-phenylalanine (10^{-4} M) or a mixture of aromatic amino acids (each at 10^{-6} M), then CAP and inducer added. There was no reversal by these supplements of the preferential inhibition of β-galactosidase synthesis. Employing the serological method of Cohn and Torriani (1951), there was no evidence for the formation of cross-reacting protein during chloramphenicol treatment. Finally, deadaptation studies (Rickenberg, et al,, 1953) showed that β-galactosidase was not degraded in bacteria growing in low concentrations of chloramphenicol.

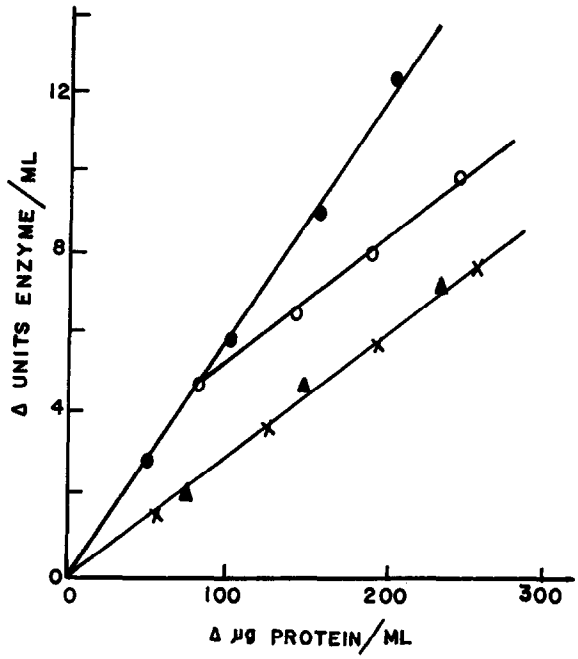

Fig. 1. The preferential inhibition of β-galactosidase synthesis when chloramphenicol is added to a growing culture of E. coli. The antibiotic (0.8 μg/ml) was added either 60 min before (▲), simultaneously (X), or 60 min after (O) the addition of inducer. The control culture is shown by (●).

It was of interest to determine the generality of this preferential inhibition of β-galactosidase synthesis with respect to other inducible (or repressible) enzymes, and those enzymes which are not inducible or repressible. Accordingly, a number of other enzymes were examined in bacteria which had been grown in low concentrations of CAP for several generations. Table I gives the results of this survey.

The data show that only inducible enzymes exhibit the phenomenon of preferential inhibition by chloramphenicol. The results with D-serine deaminase indicate that not all inducible enzymes exhibit the phenomenon. However, the significant finding is that the synthesis of enzymes which are not under repressor control (those not inducible or repressible) is not preferentially inhibited in this system.

Similar results have been obtained with puromycin, tetracycline and streptomycin, which indicates that chloramphenicol does not act in some

TABLE I

The effect of chloramphenicol on the synthesis of various enzymes.

ENZYME	INDUCIBLE OR REPRESSIBLE	SPEC. ACT. #		PERCENT REDUCTION
		CONTROL	CAP*	
β-galactosidase	yes	109	35	68
Galactoside Permease	yes	6.15	2.22	64
Tryptophanase	yes	100	30	70
Citritase	yes	385	175	58
D-serine Deaminase	yes	6.5	6.5	0
Hexokinase	no	0.50	0.48	0
G-6-P Dehydrogenase	no	1.5	1.6	0
Acid phosphatase	no	48.8	48.7	0
Pyrophosphatase	no	.335	.337	0

Specific activity is expressed as the ratio of enzyme activity to protein synthesized after the addition of CAP to the culture.

* Growth rate inhibited to 50-60% of the control rate.

specific manner, e. g., by combining with or being incorporated into the inducible protein. A possible explanation, and one which we are considering experimentally, is that treatment with these antibiotics indirectly alters the regulation mechanisms for inducible enzymes. For example, we are studying the possibility that there is repressor accumulation in bacteria treated with chloramphenicol. It may be mentioned that several experimental approaches have indicated a positive correlation between the reduction of β-galactosidase synthesis and the acceleration of RNA synthesis. These data will appear in a future publication.

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