INVESTIGATION OF CHLORAMPHENICOL ACETYLTRANSFERASE FORMATION BY *Escherichia coli* K-12 CELLS AFTER CHANGES IN INTRACELLULAR CYCLIC AMP CONCENTRATION

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The effect of cylic AMP, ACTH, and glucose on formation of the enzyme chloramphenicol acetyltransferase by cells of *Escherichia coli* CSH-2/R222 and *E. coli* WZ-78/R222 (cya_{855}) was investigated. Glucose was shown to reduce synthesis of the enzyme in *E. coli* CSH-2/R222 by inducing catabolite repression; this could be overcome by 5 mM cyclic AMP and 1000 μg/ml ACTH. Synthesis of the enzyme in *E. coli* WZ-78/R222 was resistant to catabolite repression and ACTH did not stimulate the synthesis of chloramphenicol acetyltransferase by this strain.
KEY WORDS: *Escherichia coli; chloramphenicol acetyltransferase; cyclic AMP; ACTH; catabolite repression.*

Much attention is now being paid to the study of the role of cyclic AMP in the regulations of enzyme synthesis in bacteria. Investigations have shown that cyclic AMP stimulates the formation of the enzyme chloramphenicol acetyltransferase (CAT), which codes the resistance (R) factor in *Escherichia coli* [1, 3, 4]. Cyclic AMP is formed in the cells from ATP under the influence of the enzyme adenyl cyclase [9]. In higher organisms some hormones, notably ACTH, can increase adenyl cyclase activity with a consequent increase in the intracellular cyclic AMP concentration [9]. Meanwhile experiments have shown that during growth of *E. coli* on medium with glucose as the source of carbon, the intracellular cyclic AMP concentration falls [6], and this leads to a reduction in synthesis of the enzyme β-galactosidase, coded by the lactose operon, for the regulation of which an essential component is known to be cyclic AMP [7, 8].

With these facts in mind, and also remembering that ACTH stimulates production of β-galactosidase, an enzyme coded by the lactose operon of *E. coli* [2], in the investigation described below CAT production was studied under conditions modifying the intracellular concentration of cyclic AMP.

EXPERIMENTAL METHOD

Strains *E. coli* K-12: CSH-2/R222 and WZ-78/R222 were used as experimental models. Strain *E. coli* WZ-78/R222 possesses a mutation (cya_{855}) which affects the enzyme adenyl cyclase, as a result of which the intracellular concentration of cyclic AMP is lowered. CAT production was determined by Hestrin's method [5]. Fuller details of the materials and method were described previously [1]. Cyclic AMP (Fluka, West Germany) and ACTH generously provided by F. Yu. Ryshka (All-Union Research Institute of Technology of Blood Substitutes and Hormonal Preparations) were used in the experiments. The hormone was isolated by Ryshka from pig pituitary glands. Preliminary experiments showed that, in a concentration of 1000 μg/ml (40 units/ml) the ACTH had the most powerful stimulating effect on CAT production in *E coli* CSH-2/R222.

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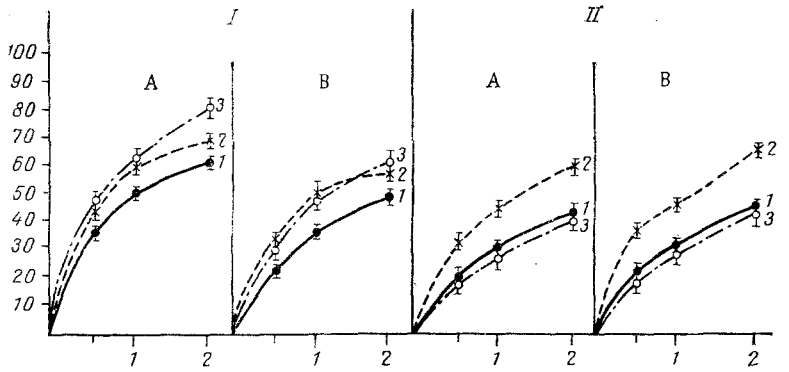

Fig. 1. Dynamics of inactivation of chloramphenicol by cells of *E. coli* CSH-2/R222 and *E. coli* WZ-78/R222: A) investigation of formation of CAT during growth of cultures of *E. coli* CSH-2/R222 and *E. coli* WZ-78/R222 on medium with mannitol; B) investigation of CAT formation during growth of cultures of *E. coli* CSH-2/R22 and *E. coli* WZ-78/R222 on medium with glucose. I) *E. coli* CSH-2/R222: 1) *E. Coli* CSH-2/R222, 2) *E. coli* CSH-2/R222 + 5 mM cyclic AMP, 3) *E. coli* CSH-2/R222 + 1000 μg/ml ACTH; II) *E. coli* WZ-78/R222: 1) *E coli* WZ-78/R222, 2) *E. coli* WZ-78/R222 + 5 mM cyclic AMP, 3) *E. coli* WZ-78/R222 + 1000 μg/ml ACTH. Abscissa, time of chloramphenicol inactivation (in h); ordinate, percentage inactivation of chloramphenicol.

EXPERIMENTAL RESULTS

The results are shown as curves reflecting the dynamics of inactivation of chloramphenicol by bacterial cells of the strains studied (Fig. 1).

As Fig. 1 shows, during growth of *E. coli* CSH-2/R222 on medium with 0.4% glucose as the source of carbon, CAT production was lower than by cells of strain *E. coli* CSH-2/R222 grown on medium with 0.4% mannitol. Strain *E. coli* WZ-78/R222 was resistant to repression of CAT production by glucose in strain *E. coli* CSH-2/R222. It must be noted that production of the enzyme by *E. coli* CSH-2/R222 on medium with glucose fell to the level of CAT production by *E. coli* WZ-78/R222 which is characterized by reduced synthesis of the enzyme [1].

Cyclic AMP in a final concentration of 5 mM and ACTH in a concentration of 1000 μg/ml restored the level of CAT synthesis by *E. coli* CSH-2/R222 on medium with glucose to the level observed on medium with mannitol. Cyclic AMP and ACTH in the same doses stimulated CAT production by strain *E. coli* CSH-2/R222 grown on medium with mannitol, but their effect under these conditions was weaker.

Cyclic AMP (5 mM) considerably increased CAT synthesis by *E. coli* WZ-78/R222 on medium both with glucose and with mannitol. However, under these conditions ACTH was unable to stimulate production of the enzyme by this strain.

It can be concluded from an analysis of these results that cyclic AMP is required for CAT formation, for if the intracellular cyclic AMP concentration is lowered — in *E. coli* CSH-2/R222 as a result of growth of glucose and in *E. coli* WZ-78/R222 as a result of disturbances affecting adenyl cyclase — CAT formation is reduced and can be restored by the addition of exogenous cyclic AMP. It can also be postulated that stimulation of CAT production by ACTH in *E coli* CSH-2/R222 may perhaps be linked with activation of adenyl cyclase, for the hormone had no stimulant effect on strain *E. coli* WZ-78/R222, effective for adenyl cyclase.

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