STIMULATION OF CHLORAMPHENICOL ACETYLTRANSFERASE SYNTHESIS BY CYCLIC AMP IN BACTERIAL CELL SYSTEMS

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The effect of cyclic 3',5'-adenosine monophosphate (cAMP) on production of the enzyme chloramphenicol acetyltransferase (CAT) by whole bacterial cells was studied in strains Escherichia coli CSH-2/R222 and WZ-78/R222 (cya_{855}). CAT synthesis in strain E. coli WZ-78/R222 was shown to have an intensity only half as great as that of strain E. coli CSH-2/R222. The production of CAT by strain E. coli CSH-2/R222 was increased only very slightly by cAMP, but its effect on the production of this enzyme in strain WZ-78/R222 was appreciable.

KEY WORDS: cyclic AMP; chloramphenicol acetyltransferase; resistance to antibiotics factor (R factor); Escherichia coli.

Cyclic 3',5'-adenosine monophosphate (cAMP) controls various functions in Escherichia coli [6]: It regulates the synthesis of enzymes participating in the breakdown of sugars [5]. Experiments with cell extracts of E. coli have recently shown that cAMP regulates the level of enzymes which inactivate antibiotics, namely chloramphenicol acetyltransferase (CAT) and streptomycin adenyltransferase [1, 3], which are produced by strains of E. coli carrying the factor of resistance to antibiotics (R factor).

This paper describes an investigation of the effect of cAMP on CAT production by whole cells of E. coli K-12.

EXPERIMENTAL METHOD

Strain E. coli K-12 CSH-2/R222, carrying R factor (R222), which makes the strain resistant to tetracycline, streptomycin, chloramphenicol, and sulfonamides, and strain WZ-78, possessing a mutation (cya_{855}) as a result of which the content of the enzyme adenylate cyclase in this microorganism is lowered, were used as experimental models. Because of the very low intracellular concentration of cAMP, strain E. coli WZ-78 cannot grow on minimal medium unless the medium contains cAMP or growth factors. R factor (R222) was transmitted by conjugation to strain E. coli WZ-78 from strain CSH-2/R222.

CAT production was investigated by Hestrin's method [4]. Cells from overnight broth cultures of E. coli strain CSH-2/R222 and WZ-78/R222 were suspended in medium M-9 with the addition of only 0.4% mannitol as the source of carbon or either 1 or 5 mM cAMP, after which they were incubated for 2 h at 37°C. The cells were then sedimented by centrifugation at 5000 g for 15 min and all samples were diluted with fresh medium M-9 with 0.4% mannitol, heated to 37°C, to a concentration of $7 \cdot 10^8$ cells/ml. The antibiotic chloramphenicol was added in a dose of 500 μg/ml, and the subsequent treatment followed the method described by Garber [2]. The yield of CAT was estimated from the percentage inactivation of chloramphenicol.

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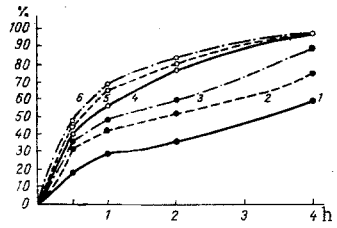

Fig. 1. Effect of cAMP on inactivation of chloramphenicol by bacterial cells: 1) E. coli strain WZ-78/R222; 2) in the presence of 1 mM cAMP; 3) the same, in the presence of 5 mM cAMP; 4) E. coli strain CSH-2/R222; 5) the same, in the presence of 1 mM cAMP; 6) the same, in the presence of 5 mM cAMP. Abscissa, time of inactivation of chloramphenicol (in h); ordinate, inactivation of chloramphenicol (in %).

EXPERIMENTAL RESULTS

The effect of cAMP on CAT production in strains E. coli CSH-2/R222 and WZ-78/R222 is illustrated in Fig. 1, which represents the dynamics of chloramphenicol inactivation by bacterial cells of the strains of E. coli studied. As Fig. 1 shows, cells of strain E. coli WZ-78/R222 produced only half the amount of CAT produced by cells of the donor strain CSH-2/R222. The addition of 1 and 5 mM cAMP caused only a very small increase, not statistically significant, in the output of CAT by strain CSH-2/R222, but in the case of strain WZ-78/R222 the production of the enzyme was increased under the influence of 1 and 5 mM cAMP by 1.5 and 1.7 times respectively in the course of 4 h. The antibiotic was inactivated by 90% in the course of 4 h, whereas without the addition of cAMP, strain E. coli WZ-78/R222 inactivated the antibiotic by only 50% in the course of 4 h.

It can be concluded from these findings that stimulation of production of the enzyme CAT in E. coli K-12 by cAMP is also observed in investigations on whole bacterial cells.

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