

INVESTIGATION OF FUNCTION OF THE LACTOSE
OPERON OF *Escherichia coli* K-12 UNDER THE INFLUENCE
OF NONSPECIFIC REGULATORS

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The possible role of cyclic AMP in the stimulating action of ACTH and hydrocortisone on the lactose operon of *Escherichia coli* K-12 was investigated. It was shown that ACTH had no effect on strains *E. coli* WZ-78/F'lac (*cya*₈₅₅) and *E. coli* CA 8001 (*L*₁), in which the system of regulation of the function of the lactose operon by cyclic AMP is disturbed. Meanwhile this hormone stimulates the lactose operon in wild-type strains: *E. coli* 200 PS/F'lac and *E. coli* 3000. Hydrocortisone stimulates the function of the lactose operon both in the wild-type strain *E. coli* 3000 and in the mutant *E. coli* CA 8001 (*L*₁). It is considered that the stimulating action of ACTH on the lactose operon is mediated through cyclic AMP and that hydrocortisone stimulates the function of the lactose operon independently of cyclic AMP.

KEY WORDS: *Escherichia coli*; lactose operon; cyclic AMP; ACTH; hydrocortisone.

The role of cyclic AMP in the regulation of the function of the lactose operon in *Escherichia coli* is now well established [5]. It has also been shown that ACTH and hydrocortisone accelerate the production of the enzyme β -galactosidase, coded by the lactose operon of *E. coli* K-12 [2]. Meanwhile, in higher organisms, some hormones, in particular ACTH, increase the activity of the enzyme adenylate cyclase, thereby increasing the intracellular concentration of cyclic AMP [6].

With these considerations in mind, it was decided to study the role of cyclic AMP in the accelerating action of ACTH and hydrocortisone on the expression of the lactose operon in *E. coli* K-12.

EXPERIMENTAL METHOD

The test objects were strains of *E. coli* K-12: *E. coli* WZ-78/F'lac and *E. coli* CA 8001. Strain *E. coli* WZ-78/F'lac possesses the *cya*₈₅₅ mutation, affecting the enzyme adenylate cyclase, as a result of which the intracellular concentration of cyclic AMP in this particular microorganism is reduced [1]. The F'lac factor was introduced into strain *E. coli* WZ-78 from *E. coli* 200 PS/F'lac by conjugation. Strain *E. coli* CA 8001 is a derivative of strain *E. coli* 3000 possessing the *L*₁ deletion in the region of the promotor of the lactose operon. The *L*₁ deletion affects the region of attachment of the cyclic AMP-receptor protein activated by cyclic AMP [4]. Wild-type strains, *E. coli* 200 PS/F'lac and *E. coli* 3000, served as the control.

The level of expression of the lactose operon was estimated from the production of β -galactosidase, which was investigated by the method of Pardee, Jacob, and Monod: Hormones and cyclic AMP in the test concentrations, together with the inducer, were added to the bacterial cultures in the logarithmic phase of growth, grown in glucose-minimal M-9 medium to a concentration of $4 \cdot 10^8$ cells/ml; the samples were incubated for 30 min at 37°C [2], after which the enzyme production was determined [3].

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TABLE 1. Effect of ACTH and Cyclic AMP on Production of β -Galactosidase ($M \pm m$)

Sample	β -Galactosidase activity, units/ml/min			
	<i>E. coli</i> 200 PS/F'lac	<i>E. coli</i> WZ-78/F'lac	<i>E. coli</i> 3000	<i>E. coli</i> CA8001
Control	19,9 \pm 0,3	8,2 \pm 0,37	20,2 \pm 0,43	1,77 \pm 0,073
500 μ g/ml ACTH	24,8 \pm 0,37	8,5 \pm 0,14	24,5 \pm 0,69	1,68 \pm 0,074
P	<0,001	>0,05	<0,001	>0,05
1000 μ g/ml ACTH	29,4 \pm 0,91	8,6 \pm 0,58	27,5 \pm 0,56	1,74 \pm 0,070
P	<0,001	>0,05	<0,001	>0,05
5000 μ g/ml ACTH	25,7 \pm 1,07	8,4 \pm 1,37	14,1 \pm 1,43	1,71 \pm 0,057
P	<0,01	>0,05	<0,01	>0,05
5 mM cyclic AMP	40,6 \pm 3,03	42,2 \pm 1,52	41,9 \pm 2,61	1,70 \pm 0,075
P	<0,001	<0,001	<0,001	>0,05

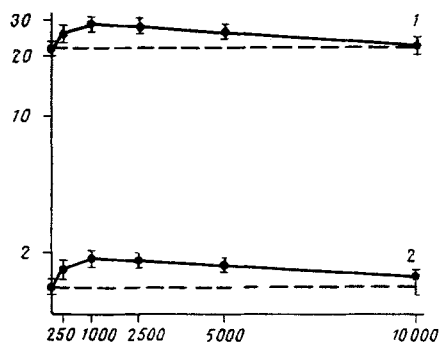


Fig. 1. Effect of hydrocortisone on β -galactosidase synthesis by strains *E. coli* 3000 (1) and *E. coli* CA 8001 (2). Abscissa, concentration of hormone (in μ g/ml; ordinate, β -galactosidase activity (in units/ml/min).

EXPERIMENTAL RESULTS

The results of investigation of the effect of cyclic AMP and ACTH on β -galactosidase production are given in Table 1. It will be clear from the results in Table 1 that expression of the F'lac factor in strain *E. coli* WZ-78/F'lac was reduced by 58% compared with expression of the F'lac factor in *E. coli* 200 PS/F'lac. The production of this enzyme by *E. coli* WZ-78/F'lac was increased fivefold, and in the wild-type strains *E. coli* 200 PS/F'lac and *E. coli* 3000 it was doubled by 5 mM cyclic AMP. Cyclic AMP had no action on the lactose operon of *E. coli* CA 8001.

ACTH caused no statistically significant changes in the levels of β -galactosidase synthesis in *E. coli* WZ-78/F'lac and *E. coli* CA 8001. Meanwhile this hormone increased production of the enzyme in the wild-type strains, its stimulating effect being maximal in a concentration in the medium of 1000 μ g/ml.

The results of the investigation of the effect of hydrocortisone on β -galactosidase synthesis in strains *E. coli* CA 8001 and *E. coli* 3000 are shown in Fig. 1. Clearly hydrocortisone stimulated β -galactosidase production both in strain *E. coli* 3000 and in strain *E. coli* CA 8001, the stimulating effect being maximal in a concentration of 1000 μ g/ml in the medium.

The results suggest that the stimulating effect of ACTH on the lactose operon is mediated through cyclic AMP as a result of interaction between the hormone and the adenylate cyclase of the bacterial cell. Because of the defect of adenylate cyclase, ACTH has no effect on strain *E. coli* WZ-78/F'lac, nor does it have an effect on *E. coli* CA 8001, because of the absence of the site of application of the stimulating effect of cyclic AMP on the lactose operon, as a result of the L_1 deletion.

Hydrocortisone is unable to interact with the adenylate cyclase of the cells of higher organisms [6]. It will be clear from the results of the present investigation that this hormone stimulates β -galactosidase production independently of cyclic AMP, since it has an action on *E. coli* CA 8001.

Hydrocortisone and ACTH thus have different mechanisms of stimulating action on the lactose operon. Further experiments must be carried out in order to prove the hypothesis expressed above regarding the mechanism of the effect of ACTH on the lactose operon.

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