ROLE OF SUBSTRATE INDUCERS IN THE MECHANISM OF ACTION OF CORTISONE ON β-GALACTOSIDASE ACTIVITY IN VARIOUS STRAINS OF Escherichia coli K-12 AND IN RAT LIVER

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UDC 576.851.38.098.31.095.18:615.357.453+ 612.351.11.014.46:615.357.453

A comparative study of the effect of cortisone on β-galactosidase synthesis in strains of Escherichia coli K-12 with an induced (E. coli 200 PS/F lac), a constitutive (E. coli ML-308), and a superrepressed (E. coli 2000 i^S) type of enzyme synthesis and in rat liver cells showed that the hormone itself has no derepressive effect. In all cases an increase in β-galactosidase synthesis takes place only in the presence of the specific substrate inducers. It is suggested that the principal stage in the mechanism of action of cortisone on the lactose operon of E. coli and on enzyme production in rat liver cells is the preliminary derepression of regions of the genome through substrate inducers.

KEY WORDS: substrate induction; cortisone; β-galactosidase; E. coli K-12; rat liver.

Elucidation of the mechanism of the effect of steroid hormones on the genome is an important problem in modern molecular biology and genetics. However, when the level of biosynthesis of RNA and protein molecules is determined by the usual methods only an overall idea of genetic activity can be obtained without allowing for any special features of the function and mechanisms of depression of individual operons [1].

The object of this investigation was to study the effect of cortisone on the function of the lactose operon of some strains of E. coli K-12 and of rat liver cells in the presence and absence of substrate inducers.

EXPERIMENTAL METHOD

The test for evaluating the effect of cortisone on the function of the lactose operon was determination of β-galactosidase activity in strains E. coli 200 PS/F lac, ML-308, and 2000 i^S by the method of Pardee et al. [3] in rat liver homogenates treated with Triton X-100, in order to detect the total activity of the enzyme [4].

The strains of microorganisms used differ from one another in the character of function of their lactose operon. In strain E. coli 200 PS/F lac enzyme synthesis is of the induced type and β-galactosidase production can be detected only after the addition of lactose or of artificially synthesized thiogalactosides to the medium. In the present experiments, when microorganisms were used as the model, the inducer was isopropylthio β-D-galactoside (IPTG) in a final concentration of 10^{-3} M. Strain E. coli Ml-308, as a result of mutation of the regulator gene of the lactose operon, produces a defective repressor, and β-galactosidase synthesis is constitutive in type. The superrepressed strain E. coli 2000 i^S has a permanent block of the operator, and consequently the lactose operon is not activated by the addition of substrate inducers to the medium. In these experiments the hormones were added in a concentration giving maximal stimulating action on β-galactosidase production [2].

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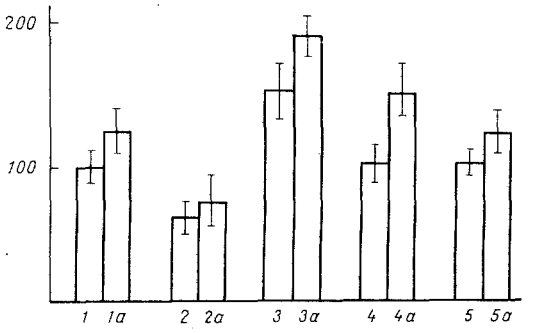

Fig. 1. Effect of cortisone on β-galactosidase activity in E. coli cultures and rat liver homogenates: 1) rats on ordinary diet (control); 1a) the same + cortisone; 2) rats receiving meat broth; 2a) the same + cortisone; 3) rats receiving milk; 3a) the same + cortisone; 4) E. coli 200 PS/F lac; 4a) the same + cortisone; 5) E. coli ML-308; 5a) the same + cortisone. Ordinate, β-galactosidase activity (in % of control).

To study the effect of cortisone on β-galactosidase synthesis in the presence and absence of substrate induction in mammals, 84 noninbred male rats weighing 150-180 g were used. The animals were divided into three groups with 28 rats in each group. The animals of group 1 were kept on an ordinary diet of food in pellet form and vegetables for 7 days. The rats of group 2 received meat broth without lactose for 7 days. The rats of group 3 received the ordinary diet but with milk additionally instead of water. Before the experiments each group of animals was divided in two equal subgroups. The rats of one of the subgroups of each group received an intraperitoneal injection of cortisone in a dose of 5 mg/kg (two injections at intervals of 12 h). The animals were killed 24 h after the 2nd injection and the total β-galactosidase activity in homogenate of their liver was determined.

EXPERIMENTAL RESULTS

The experiments showed that addition of the hormone to a culture of E. coli 200 PS/F lac in the absence of the inducer does not affect β-galactosidase synthesis. Meanwhile, a combined action of cortisone and IPTG caused changes in β-galactosidase production. For instance, whereas the mean activity of the enzyme after addition of IPTG alone averaged 17 units/ml/min (100%), when the glucocorticoid concentration in the medium was 500 μg/ml activity was increased to 26 units/ml/min (152%). An increase in the quantity of hormone in the culture to 10,000 μg/ml blocked enzyme synthesis.

Addition of cortisone alone in a concentration of 1000 μg/ml to a culture of strain ML-308 with the constitutive type of β-galactosidase synthesis increased enzyme production from 36 units/ml/min (100%) to 44 units/ml/min (122%). A further increase in the hormone concentration in the culture to 10,000 μg/ml led to a decrease in β-galactosidase production to 23 units/ml/min.

In the superrepressed system (strain E. coli 2000 i^S) cortisone was unable to influence β-galactosidase synthesis regardless of its concentration or of the presence of substrate inducer (IPTG) in the culture.

Consequently, the results showed that when strains of E. coli K-12 were used as the model, cortisone in a certain concentration could accelerate β-galactosidase synthesis in an induced system, but only in the presence of substrate inducer. It is very interesting that the glucocorticoid could increase enzyme production in the culture of the constitutive strain also (Fig. 1). This fact shows that the important stages in the mechanisms of regulation of enzyme activity are those not connected with direct derepression of the lactose operon, but which determine posttranscription interactions.

In the light of these observations the possible role of substrate induction in the regulation of β-galactosidase synthesis under the influence of cortisone was studied in rat liver cells. It was found that in animals kept on the ordinary laboratory diet administration of cortisone caused ill-defined but statistically significant increases in enzyme activity. Keeping the animals for 7 days on a diet providing no lactose whatsoever (meat broth) led to a marked fall in the control level of β-galactosidase; injections of cortisone into these rats, moreover, did not stimulate production of the enzyme. Conversely, in rats receiving a diet rich in lactose, a high level of β-galactosidase was found and they reacted to injection of cortisone by a considerable and statistically significant increase in enzyme activity (Fig. 1).

The results suggest that the mechanisms of action of cortisone on the lactose operon of E. coli and on enzyme production in rat liver cells incorporate identical stages, and that the most important of these stages is preliminary derepression of regions of the genome by substrate inducers.

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ISOLATION OF HIGH-MOLECULAR-WEIGHT INFECTIOUS DNA FROM TYPE 1 HERPES SIMPLEX VIRUS

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UDC 576.858.13.(Herpes).098.396.332

Isolation of DNA from type 1 herpes simplex virus (strain L2) is described; the DNA possessed the characteristics of an intact molecule: sedimentation rate, physical length, and infectivity. Data on infectivity of preparations of this DNA were obtained in cultures of chick embryonic fibroblasts.

KEY WORDS: herpes simplex virus; infectious DNA.

Current interest in the DNA of herpes viruses is due to the recently discovered possibility that the virus genome can be mapped by the use of restriction endonucleases, and in the case of the herpes viruses this could show the region of the molecule responsible for the oncogenic properties of some members of this group. Following such an investigation on the shorter DNAs of papova viruses and adenoviruses [8, 9, 11], this could shed some light on the question of virus carcinogenesis. This same approach to the study of virus DNA has a more general importance. It is evident that the preferred object for investigations of this sort must be the intact DNA molecule. In herpes viruses it has a molecular weight of about 10^8 daltons [5]. The isolation of such long molecules, incorporating as they do ribonucleoproteins [3, 7], is rendered more difficult by their high sensitivity to various external factors and, in particular, mechanical injury, leading to fragmentation during isolation.

The object of this investigation was to obtain preparations of intact DNA molecules from strain L2 of type 1 herpes simplex virus, which itself was isolated earlier in the writers' laboratory [1], and to assess their nativeness. A factor of considerable importance in this mater could be that as the system for reproduction and titration of the virus and also for assessment of the infectivity of its DNA a culture of chick embryonic fibroblasts (CEF) was used, although these cells are usually insensitive to type 1 herpes virus; it must, admittedly, be remembered that strain L2 is specially adapted to these cells. The criteria of nativeness of the DNA obtained were its sedimentation rate in a glycerol density gradient, electron-microscopic analysis of the preparation, and its infectivity.

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. D. Solov'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 1, pp. 26-28, January, 1977. Original article submitted January 21, 1976.