Control of Basal Level Activity of $\beta$-Galactosidase in *Escherichia coli*

**PETER OVERATH**

Institut für Genetik der Universität zu Köln, Köln-Lindenthal

Received January 30, 1968

Summary. *E. coli* 15 and its derivatives contain about ten times more $\beta$-galactosidase in the absence of inducer than *K12* or *B*-strains. The maximally induced level is the same in all strains. The elevated basal-level of enzyme in 15-strains is not inhibited by $\alpha$-nitrophenyl-$\beta$-D-fucoside. The repressor of the lac-operon seems to have the same affinity for the inducer in all strains. Functional analysis reveals that the higher basal-level is due to the $i$-gene product of 15-strains. It is concluded that 15-strains contain either an altered repressor with lower affinity for the operator or a smaller amount of repressor than *K12*-strains. The data are discussed in relation to the problem of the control of repressor formation.

A. Introduction

A variety of genetic, physiological and biochemical experiments indicate that the rate of synthesis of $\beta$-galactosidase in *Escherichia coli* is regulated by an internal repressor which is antagonized by the addition of specific inducers (Pardee, Jacob and Monod, 1959; Jacob and Monod, 1961; Sadler and Novick, 1965; Gilbert and Muller-Hill, 1966). Relatively little is known however about what determines the maximal level of enzyme in the presence of a saturating concentration of inducer and the minimal level in the absence of any inducer. The maximal expression of the lac-operon is dependent on a genetic region between the operator (o) and the structural gene for $\beta$-galactosidase (z) which was called the promoter (p) (Jacob, Ullman and Monod, 1964; Scaife and Beckwith, 1966). The structure of the promoter might therefore give an ultimate answer to the question of maximal enzyme production. The minimal amount of enzyme formed in a growing culture, the basal level, has received little attention. An extension of experiments carried out by Hanawalt and Wax (1964) seemed to show that there exists no special mechanism which couples repressed enzyme production with DNA-replication (Overath and Stange, 1966). In accord with these findings Fangman, Gross and Novick (1967) showed in a recent communication that repressed enzyme is produced at the same rate as total protein, when DNA-synthesis is stopped by a shift in temperature in a mutant with thermosensitive DNA-synthesis. One plausible explanation for the presence of a basal level of enzyme would be that the effectiveness and/or concentration of the repressor (the $i$-gene product) is not sufficient to cause a complete cessation of enzyme synthesis. Analysis of basal level production should therefore shed some light on the question of the control of repressor formation.

It has been found (Overath and Stange, 1966) that *E. coli* 15 contains a higher basal level of $\beta$-galactosidase than *K12*-strains whereas the maximally induced level is essentially the same in all strains. If the gene(s) responsible for the
maximum production of enzyme are assumed to be identical in both strains, at least four possibilities may be considered for the difference in basal level synthesis:

1. The operator of the lac-operon in 15-strains differs from the operator of K12-strains in having less affinity for the repressor.

2. The concentration of active repressor present in 15-strains is lower than in K12-strains. This could be due (a) to a smaller amount of repressor produced by the i-gene or (b) to the presence of an internal inducer in 15-strains which causes partial induction.

3. The repressor elaborated by 15-strains differs from the one in K12-strains in having less affinity for the operator. The following experiments exclude the possibilities stated in 1 and 2b.

While the data do not strictly exclude a lowered affinity of the repressor for the operator, a lowered repressor concentration in 15-strains is considered to be the most likely explanation for the altered basal level of enzyme. A preliminary report of this work has been presented (OVERATH, 1967).

B. Materials and Methods

I. Reagents

IPTG and ONPG were obtained from Mann Research Laboratories, New York, USA, TMG and melibiose from Calbiochem, Los Angeles, Calif., 14C-TMG from New England Nuclear Corp., Boston, Mass. and NG from Ferak, Berlin, Germany. ONPF was synthesized from D-fucose (Fluka, Basel, Switzerland) according to BECK (1962). The recrystallized substance had a melting point of 183—185°C.

II. Bacteria

The strains of Escherichia coli used in this paper are listed in Table 1. I would like to thank all the authors listed for providing these strains.

III. Media

The following media were used: nutrient broth (ADELBerg, MANDEL and CHEEN CHEN, 1965), nutrient agar (nutrient broth + 1.5 % agar), penassay-broth (17.5 g Difco Bacto Antibiotic Medium 3 per liter), EMB-indicator plates (LEDERBERG, 1950) with or without 100 μg streptomycin per ml, minimal plates (LENGELER, 1966) with 0.2 % sugars, and when necessary, per ml, 20 μg amino acids of the L-form, 2 μg thymidine, 10 μg uracil, 5 μg vitamin B1. For the assays of β-galactosidase and TMG-permease I all strains were grown in the following medium: mineral solution “56” (MOKOD, COHEN-BAZIRE and CORN, 1951) + 0.2 % glycerol, 0.1 % casamino acids (Difco, vitamin-free) and, per ml, 2 μg thymidine, 10 μg uracil, 40 μg DL-threonine, 20 μg L-leucine, 20 μg L-methionine, 20 μg arginine, 5 μg vitamin B1.

IV. Isolation of Mutants

In most cases an overnight culture of cells in nutrient broth was mutagenized by treatment with nitrous acid to about 0.1 % survival (KAUDEWITZ, 1959). The cells were then plated directly on EMB-lactose-plates. In some cases, for instance with the rec-strain, NG was used as a mutagen (ADELBerg et al., 1965). The lac-mutants obtained were characterized by determination of β-galactosidase and TMG-permease I all strains were grown in the following medium: mineral solution “56” (MOKOD, COHEN-BAZIRE and CORN, 1951) + 0.2 % glycerol, 0.1 % casamino acids (Difco, vitamin-free) and, per ml, 2 μg thymidine, 10 μg uracil, 40 μg DL-threonine, 20 μg L-leucine, 20 μg L-methionine, 20 μg arginine, 5 μg vitamin B1.

Abbreviations used: IPTG, isopropyl-β-D-thiogalactoside; ONPG, o-nitrophenyl-β-D-galactoside; TMG, methyl-β-D-thiogalactoside; NG, N-methyl-N'-nitro-N-nitroso-guanidine; ONPF, o-nitrophenyl-β-D-fucoside.

I thank Dr. K. Herrmann, Freiburg i. Br. for his advice in preparing this substance.