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The Preferential Synthesis of β -Galactosidase in *Escherichia coli*

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SUMMARY: The induced formation of β -galactosidase in relation to overall protein synthesis in *Escherichia coli* was examined under conditions where the enzyme inducer was required for growth. There was, initially, a substantial production of β -galactosidase without any detectable increase in total cell protein. This phenomenon has been termed 'preferential' β -galactosidase synthesis. The occurrence of preferential β -galactosidase synthesis indicates that β -galactosidase formation is a discrete event in cellular metabolism, and is not necessarily geared to overall cell growth. The data obtained also suggest that the carbon source(s) for preferential β -galactosidase synthesis might be of endogenous origin.

It has been amply demonstrated (e.g. Baron, Spiegelman & Quastler, 1953; Knox & Pollock, 1944; Stephenson & Stickland, 1933) that enzyme formation can occur in non-viable cells, or under conditions where environmental limitations are placed on cell proliferation. However, while these demonstrations dissociate protein formation from cell division, they do not indicate the relationship between the formation of one protein and that of other cell proteins. The work of Monod, Pappenheimer & Cohen-Bazire (1952), with *Escherichia coli*, suggests a close integration between β -galactosidase formation and the formation of other cell proteins. They found that when β -galactosidase formation was induced during growth under conditions where the enzyme was not essential for the growth of the culture, the enzyme was formed as a constant proportion of the total protein synthesized by the culture. However, earlier observations (Lester, 1951) indicated that under other conditions, where β -galactosidase was required for growth, a constant proportionality between the formation of β -galactosidase and total cell material was not apparent. A similar situation has been noted by Benzer (1953). It is the purpose of this paper to examine further the relationship between the synthesis of β -galactosidase and that of overall protein synthesis in *E. coli*.

METHODS

Strains and medium. The strains used in these experiments were *Escherichia coli*, strain W-1485, which has no growth factor requirements and is non-lysogenic, and *E. coli* strain Y-Mel₁ which is unable to utilize melibiose for growth. Both strains were derived from *E. coli* strain K-12. A defined salts medium (Rickenberg, Yanofsky & Bonner, 1953) was used and the sugars were autoclaved separately and added after the salts solution was cooled. The cultures were grown under forced aeration at either 30° or 37°, as will be indicated.

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Preparation of cell extracts. Organisms were harvested by centrifugation, washed twice with 0.9% (w/v) NaCl solutions, and suspended in 0.05 M-sodium phosphate buffer at pH 7.0. Cell suspension (5–6 ml.) and about 3 g. Ballotini glass beads were agitated for 20 min. in a cell disintegrator (Mickle, 1948), kept at 2–3°. The disrupted cell suspensions were cleared of cell debris by centrifugation at 20,000 g for 1 hr. Supernatant solutions were assayed for β -galactosidase activity and protein.

Protein and β -galactosidase assay. The protein content of the extracts was determined by the method of Lowry, Rosebrough, Farr & Randall (1951), with Armour plasma fraction V as a reference standard.

β -Galactosidase activity was determined by a modification (Rickenberg *et al.* 1953) of the method described by Lederberg (1950). The reaction was carried out in a volume of 3.0 ml. with a final concentration of 0.001 M-*o*-nitrophenyl- β -galactoside (ONPG) and 0.05 M-sodium phosphate buffer (pH 7.0). After incubation at 37° for an appropriate time, depending upon the activity of the extracts, 2.0 ml. of M-K₂CO₃ were added to stop the reaction and to develop the colour of the *o*-nitrophenol released. The amount of *o*-nitrophenol released was measured in a Klett-Summerson colorimeter using the 420 m μ . (blue) filter; the values obtained were calculated for a 10 min. incubation period.

RESULTS

Previous experiments (Lester, 1951) showed that when cells were cultured in a medium containing glucose + lactose appreciable amounts of β -galactosidase were formed during the growth lag between the end of growth on glucose and the onset of growth on lactose. However, β -galactosidase synthesis was measured as a function of time, and its relationship to the overall increase in protein was not determined. The following experiment was carried out to examine enzyme formation as a function of total protein synthesis. Glucose-grown W-1485 cells were inoculated into 3.0 l. medium containing 0.03% (w/v) glucose + 0.2% (w/v) lactose and the culture incubated at 30°. Samples of known volume were withdrawn at intervals, and the cells harvested in the cold and extracted. The extracts were assayed for protein content and β -galactosidase activity.

The relationship between overall protein formation and β -galactosidase synthesis is illustrated in Fig. 1. It can be seen that during the glucose phase of growth (0–5.5 hr.) little enzyme was formed. Then, during the diauxic lag (5.5–7.0 hr.), when no increase in total protein was detected, a sharp increase in β -galactosidase activity was observed. When growth resumed, on lactose, β -galactosidase was formed as a constant proportion of overall protein synthesis. Here, in the presence of the same inducer, the relationship between β -galactosidase formation and overall protein formation differs considerably. For an equal amount of β -galactosidase, the net protein formed (if any) during the lag phase does not nearly correspond to the net protein formed during exponential growth. This formation of β -galactosidase during the lag phase is termed 'preferential' synthesis.

Fig. 1

Fig. 2

Fig. 3

Figs. 1-3. Relationship between protein and β -galactosidase activity. The values given are for the extract obtained from 1.0 ml. of culture. The numbers in the figures indicate the age of the cultures. Fig. 1. *Escherichia coli* strain W-1485 cultured in a glucose+lactose medium. Fig. 2. *E. coli* strain W-1485 (●) and strain Y-Mel⁻ (○) cultured in a glucose+melibiose medium. Fig. 3. *E. coli* strain W-1485 cultured in lactose (●) and in maltose+lactose (○) media.

The formation of β -galactosidase in the absence of overall cell growth poses a question concerning the sources of energy and carbon for β -galactosidase synthesis during the diauxic lag. Is the low level of β -galactosidase activity found during growth on glucose adequate for the utilization of lactose for β -galactosidase formation? Or does residual glucose or some endogenous material yield sufficient energy and carbon for the preferential synthesis of β -galactosidase? In order to examine this question use was made of *Escherichia coli* strain Y-Mel₁⁻.

Melibiose has been shown to be an excellent inducer of β -galactosidase formation, and cells grown on melibiose exhibit 1.5 to 2.0 times as much β -galactosidase as those grown on lactose (Monod, Cohen-Bazire & Cohn, 1951; Lester & Bonner, 1952). Strain Y-Mel₁⁻ cannot utilize melibiose, but when grown in the presence of melibiose and certain utilizable carbon sources, it will form β -galactosidase. Just as in strain W-1485, glucose inhibits β -galactosidase formation in strain Y-Mel₁⁻. Consequently, when strain Y-Mel₁⁻ is grown on a medium containing glucose + melibiose, β -galactosidase should not be formed unless glucose or some form of endogenous material is available for β -galactosidase formation. Since strain W-1485 exhibits a longer diauxic lag with glucose + melibiose than with glucose + lactose it was also of interest to determine whether preferential synthesis of β -galactosidase might also be more pronounced. Therefore, the protein content and β -galactosidase activity of strains Y-Mel₁⁻ and W-1485 were examined during growth in a glucose-melibiose medium.

Using glucose-grown strains W-1485 and Y-Mel₁⁻ as inocula an experiment similar to the one previously described was carried out. The concentrations of sugars used were 0.03 % (w/v) glucose + 0.1 % (w/v) melibiose. The relationship between total protein and β -galactosidase is shown in Fig. 2. The results obtained in the experiment with strain W-1485 resemble those observed on glucose + lactose. In this case the duration of the diauxic lag was about 2.0 hr., and a larger amount of β -galactosidase was produced during this period while no appreciable protein formation was observed. During subsequent growth on melibiose the enzyme was produced as a constant proportion of total protein synthesis. It may be noted that β -galactosidase forms a larger proportion of the protein synthesized than in the case of lactose, which is in accord with the aforementioned observations that melibiose is a better inducer of β -galactosidase formation than lactose.

When strain Y-Mel₁⁻ was used, β -galactosidase formation began when growth and overall protein formation ceased. Although the enzyme was formed at a slower rate than with strain W-1485 (requiring 6.5 hr. to reach the same activity as reached by strain W-1485 in 2.0 hr.) no growth was observed. Since strain Y-Mel₁⁻ was unable to utilize melibiose for growth, the β -galactosidase presumably was synthesized from some carbon source other than the inducer.

The experiments presented above argue against the utilization of the inducer as a carbon source for preferential β -galactosidase formation. However, the question of the exogenous or endogenous nature of the source of material for

β -galactosidase formation remains. When maltose-grown cells are used to inoculate a medium containing maltose + lactose, maltose can be immediately utilized as a carbon and energy source, and β -galactosidase would not be required for the synthesis of cell protein. On the other hand, when maltose-grown cells are used to inoculate a medium containing only lactose as a carbon source, β -galactosidase will be required for cell growth. The use of a well washed inoculum decreases the possible presence of such exogenous materials as might have been present under diauxic conditions. Strain W-1485 was grown on medium containing 0.1% (w/v) maltose, the organisms harvested after growth had ceased, and washed three times with saline. A concentrated suspension of maltose-grown organisms was used to inoculate media containing 0.2% (w/v) lactose + 0.2% (w/v) maltose, and 0.2% (w/v) lactose alone. The sampling and culture conditions were as described earlier, except that the incubation temperature was 37°. The relationship between total protein and β -galactosidase activity of the extracts is illustrated in Fig. 3. It may be noted here that protein synthesis began almost immediately in the culture where maltose + lactose were present. Where lactose only was present no increase in protein was observed during the first hour of culture. When maltose + lactose were present, β -galactosidase was formed as a constant proportion of total protein synthesized. This is in agreement with the results obtained by Monod *et al.* (1952) under similar conditions.

When lactose alone was present β -galactosidase formation again took place without any detectable increase in protein. Since washed cells, which had ceased growing on a medium limiting in maltose, were used as inoculum, and since the utilization of the inducer for the preferential synthesis of the enzyme was indicated as being unlikely by the previous experiment, it is reasonable to assume that the materials for preferential β -galactosidase synthesis were of endogenous origin.

DISCUSSION

The experiments described show that, under conditions where β -galactosidase is required for growth, large amounts of β -galactosidase (approximately one hundred times the basal activity) can be produced without any detectable increase in total cell protein. Following the phase of preferential synthesis the relationship between total protein formation and β -galactosidase formation is constant; such 'proportional' synthesis is also observed under conditions where the inducer is not required for growth. Since proportional β -galactosidase synthesis occurs when the culture is actively growing, this type of synthesis would seem to be a reflexion of growth conditions rather than of a basic characteristic of protein synthesis. The preferential synthesis of β -galactosidase appears to be largely independent of the synthesis of other cell proteins, and might indicate the absence of common pathways for the synthesis of proteins in *Escherichia coli*. Thus, the formation of β -galactosidase might well be, primarily, a discrete synthetic event. These experiments strongly indicate the utilization of endogenous material for preferential β -galactosidase synthesis; however, they do not permit one to ascertain the nature of these materials. Monod & Cohn

(1953) and Rotman & Spiegelman (1954) found, under conditions where the inducer was not required as a carbon source, that pre-existing cell proteins did not appear to serve as precursors of β -galactosidase. Consequently, the possibility of protein interconversion in preferential β -galactosidase synthesis might seem unlikely. However, the conclusions drawn from experiments in which the inducer is not required for growth do not necessarily obtain for preferential synthesis, which is observed when the inducer is required for growth. The exclusiveness of preferential β -galactosidase synthesis, and the induction of β -galactosidase formation by the melibiose-less mutant, might argue for the utilization of endogenous material for preferential synthesis.

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