β-Galactosidase of Streptococcus lactis

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Received for publication 24 September 1964

ABSTRACT

CITTI, J. E. (Oregon State University, Corvallis), W. E. SANDINE, AND P. R. ELLIKER. β-Galactosidase of Streptococcus lactis. J. Bacteriol. 89:937-942. 1965.—Synthesis of β-galactosidase by several strains of Streptococcus lactis was induced by lactose. The rate of hydrolysis of o-nitrophenyl-β-D-galactopyranoside was used to measure enzyme activity. The enzyme of all but one strain was unstable when whole cells were sonicated or treated with toluene; the enzyme of one strain of S. lactis was stable to these treatments, which resulted in at least a fivefold increase in activity over that found in whole cells. The optimal assay conditions for toluene-treated cells of this strain involved incubation at 37°C in pH 7.0 sodium phosphate buffer. Lactose was the most effective inducer of enzyme synthesis. Methyl-β-D-thiogalactopyranoside, iso-propyl-β-D-thiogalactopyranoside, and galactose were also inducers of the enzyme, but were not as effective as lactose. Melibiose, maltose, and calcium lactobionate were poor inducers of enzyme synthesis. Exogenously supplied glucose repressed enzyme synthesis. The means of control of induced β-galactosidase synthesis in S. lactis was similar to that in Escherichia coli.

β-Galactosidase in microorganisms has long served as a model system for the study of induced enzyme synthesis. For example, Jacob and Monod (1961) developed their hypothesis for the genetic control of enzyme synthesis from results obtained during studies with strains of Escherichia coli in which β-galactosidase production was inducible. This enzyme also has been reported to be inducible in other microorganisms, including Paracolobactrum aerogenoides (Anderson and Rickenberg, 1960), Shigella sonnei (Rickenberg, 1960), Staphylococcus aureus (McClatchy and Rosenblum, 1963), and Bacillus megaterium (Landman, 1957).

β-Galactosidase has been demonstrated in one strain of Streptococcus lactis (Yakil and Shahani, 1962), but no biochemical or physiological properties of the enzyme have been reported. The objectives of the present study were to develop a system for the study of β-galactosidase synthesis in S. lactis and to determine some properties of the enzyme.

MATERIALS AND METHODS

Microorganisms. Several strains of S. lactis were used during the initial stage of this work. S. lactis C2, C10, and E were obtained from the culture collection of the Department of Microbiology at Oregon State University, Corvallis, and S. lactis 7962, 7963, and 11454 were obtained from the American Type Culture Collection, Washington, D.C. Cultural and biochemical characteristics of these organisms have been reported by Sandine et al. (1962). As discussed below, only S. lactis 7962 possessed the desired β-galactosidase properties, and this organism was used throughout most of this study. A strain of E. coli B obtained from the Department of Microbiology, Oregon State University, was also included during assays for β-galactosidase as a comparative control. All cultures were maintained by daily transfer in a lactose broth, described below, with incubation at 32°C.

Media. Cultures were routinely propagated in a liquid medium which consisted of the following: lactose, 10 g; Difco Tryptone, 10 g; Difco yeast extract, 5 g; gelatin, 2.5 g; sodium chloride, 4 g; sodium acetate, 1.5 g; ascorbic acid, 0.5 g; and distilled water to 1.0 liter. The pH of this medium was adjusted to 7.0 prior to autoclaving. For induction experiments, the above medium was prepared without lactose, autoclaved, and cooled. A filter-sterilized solution of lactose was then added to this sugar-free broth to a concentration of 0.01 g/ml. In some experiments, maltose was added to a concentration of 0.002 g/ml.

Buffer solutions. Cells were washed, suspended, and assayed in one of the following buffer solutions: 0.05 M sodium phosphate (pH 6.0 to 8.0), 0.05 M potassium phosphate (pH 7.0), 0.05 M tris(hydroxymethyl)aminomethane (Tris) (pH 7.0), 0.05 M Tris + 0.05 M sodium chloride (pH 7.0), or 0.05 M Tris + 0.05 M sodium phosphate (pH 7.0). When indicated, MnCl₂·4H₂O was added to a concentration of 0.0004 M.

Harvesting of cells. Culture samples were immediately chilled and centrifuged at 3,000 × g
in a centrifuge refrigerated at 1 C. The harvested cells were washed twice with cold buffer.

Preparation of whole-cell suspensions. Washed cells were suspended in one of the above buffers. For whole-cell assays, suspensions of E. coli contained about 3 X 10^6 cells per milliliter, and suspensions of S. lactis strains about 3 X 10^4 cells per milliliter.

Preparation of toluene-treated cell suspensions. Washed cells were suspended in buffer solution. Suspensions of E. coli B contained approximately 3 X 10^6 cells per milliliter; S. lactis 7962, 3 X 10^6 cells per milliliter; and all other S. lactis strains, 3 X 10^4 cells/ml. Four-ml volumes of these suspensions were treated with 0.2 ml of toluene-acetone (1:9) solution, and incubated for 5 min at 25 C with vigorous agitation. The treated suspensions were immediately assayed for enzyme as described below.

Preparation of cell-free extract. Washed cells of S. lactis 7962 or S. lactis C2 were suspended in 0.05 M sodium phosphate buffer to a concentration of about 3 X 10^6 cells per milliliter, and ruptured in a Raytheon sonicator oscillator for 20 min. The cell debris was removed by centrifugation at 9,000 X g for 12 min at 1 C. The supernatant liquid was diluted 10-fold with buffer, and assayed for enzyme as outlined below.

Assay of β-galactosidase. The chromogenic substrate 0-nitrophenyl-β-D-galactopyranoside (ONPG) was used to measure enzyme activity (Lederberg, 1950). Solutions of 0.005 M ONPG were prepared in the above buffers. A 1.0-ml volume of a whole-cell suspension, toluene-treated cell suspension, or cell-free extract was incubated with 4.0 ml of ONPG solution for 15 min at, unless otherwise indicated, 37 C. Color development was stopped by adding 5.0 ml of cold 0.5 M sodium carbonate to the reaction mixture. Cells were removed from the assay mixture by centrifugation at 9,000 X g for 12 min at 1 C. The absorbancy of the supernatant liquid was measured at 420 mµ.

The units of 0-nitrophenol liberated from ONPG were determined from a standard curve, which was prepared by measuring the change in absorbancy produced by varying concentrations of 0-nitrophenol. The amount of 0-nitrophenol liberated per minute was directly proportional to the quantity of enzyme in the assay mixture. The dry weight of a cell suspension was obtained from a standard curve relating cell suspension optical density at 420 mµ to cell dry weight (Society of American Bacteriologists, 1957). The specific activity of β-galactosidase is expressed as units of 0-nitrophenol liberated from ONPG per milligram of cell dry weight per minute. One unit of enzyme is equivalent to 1 µ mole of 0-nitrophenol liberated from ONPG per minute. The differential rate of β-galactosidase induction, P, is defined as the differential rate of synthesis, and was obtained from the slope of a plot of enzyme units per milliliter against cell dry weight per milliliter.

Induction of enzyme synthesis. Lactose was used to induce β-galactosidase synthesis by the several strains of S. lactis and the one strain of E. coli. A 12-hr culture was inoculated into broth containing 0.01 g/ml of lactose. All inocula were 1%. The inoculated broth was incubated at 32 C for 10 hr. The cells were then immediately harvested and assayed for enzyme as described above. Also, 7- to 10-hr cultures of S. lactis 7962 were prepared in this manner and used to find the optimal assay pH and temperature. The inducing ability of galactose, glucose, methyl-β-D-thiogalactopyranoside (TMG), isopropyl-β-D-thiogalactopyranoside (IPTG), o-nitrophenyl-β-D-β-o-galactopyranoside (ONPG), p-nitrophenyl-β-D-β-o-galactopyranoside (PNPG), melibiose, calcium lactobionate, and borate was compared to that of lactose, with use of a modified procedure of Dénes (1960). A 12-hr culture of S. lactis 7962, which was grown at 32 C in broth containing maltose as the sole carbohydrate, served as the inoculum; 3 ml of this culture were inoculated into 297 ml of broth containing 0.002 g/ml of maltose as the sole carbohydrate. This culture was incubated for 3 hr at 32 C, and then divided into several equal portions. A filter-sterilized solution of the compound(s) to be tested was added to one of these portions to a concentration of 0.01 M, or, in the case of borate (as boric acid, pH 7.0), to 0.05 M. The cultures were incubated for an additional 4.5 to 10.5 hr. During this period, cell samples were obtained at 1.5-hr intervals, and immediately chilled, harvested, treated with tolune, and assayed for enzyme activity as described above.

Procedure for catabolite repression studies. The effect of glucose and galactose on enzyme synthesis was examined by a modified method of Dénes (1960). A 12-hr culture of S. lactis grown in maltose broth at 32 C served as the inoculum; 2 ml of this culture were inoculated into 198 ml of broth which contained 0.002 g/ml of maltose as the sole carbohydrate. After incubating for 3 hr at 32 C, the culture was divided into five portions of 40 ml each. A filter-sterilized lactose solution was added to four of these cultures to a concentration of 0.01 M. After an additional incubation period of 1.5 hr, a solution of glucose, galactose, or glucose plus galactose was added to one of three lactose-induced cultures. Each of these sugars was present at a final concentration of 0.005 M. One of the five cultures contained no lactose, glucose, or galactose, and another contained lactose, but no glucose or galactose. Samples were removed from each of the five cultures at 1.5-hr intervals throughout this experiment, and immediately chilled. The cells were harvested, treated with tolune, and assayed for enzyme as described above.

RESULTS

Enzyme activity of lactose-induced cells. The β-galactosidase-specific activities of whole-cell suspensions and toluene-treated cell suspensions of various strains of S. lactis and one strain of E. coli are presented in Table 1. The specific activity of enzyme of untreated cells of the S. lactis
strains ranged from 0.014 to 0.050. The enzyme in cells of E. coli B, assayed in the same manner, had a specific activity of 0.40; however, this activity increased more than 10-fold after toluene treatment. The β-galactosidase activity of all but one strain of S. lactis was destroyed upon toluene treatment. Manganese ion (as 0.0004 M MnCl₂·4H₂O), which is known to stabilize the β-galactosidase in S. faecium (Buecher and Brock, 1962), did not stabilize the enzyme in the other strains. Neither decreasing the time of toluene treatment from 5 min to 1 min, nor reducing the assay incubation temperature from 37 to 30 or 25°C affected the apparent unstable nature of the enzyme in these S. lactis strains. In a follow-up study, cell suspensions of 36 strains of S. cremoris were assayed for β-galactosidase in this manner. None of these strains produced an enzyme stable to toluene treatment. The enzyme in S. lactis 7962, however, was stable and about 10 times more active after toluene treatment. Increased toluene treatment times of up to 20 min did not change the activity of the enzyme in this organism. Although the cells were no longer viable after toluene treatment, microscopic examination revealed no cell lysis. The enzyme in this strain was also stable in cell-free extracts prepared by sonic treatment; however, based on equal numbers of cells, its specific activity (0.43) was nearly one half that of toluene-treated cells (0.77). In contrast, stable enzyme in cell-free extracts of S. lactis C2 could not be prepared by sonic treatment, disruption in a French press, or grinding with alumina or extra-fine glass beads. Neither Mn⁺⁺ nor reduced glutathione (0.0005 M) stabilized the enzyme in these preparations. S. lactis C2 was also sonic-treated in the presence of lactose, the natural substrate of the enzyme. The cell-free extract was passed through a Sepha-

dex G-50 column to remove the lactose just prior to enzyme assay. Enzyme stability also could not be preserved by this procedure.

Optimal enzyme assay conditions. Lactose-induced cells of S. lactis 7962 were used to determine the optimal β-galactosidase assay conditions. The effect of assay incubation temperature on enzyme activity is shown in Fig. 1. The activity of toluene-treated cells increased with temperature up to 40°C, and then dropped sharply. A temperature of 37°C was used for the routine assay of β-galactosidase. In contrast to toluene-treated cells, untreated whole cells exhibited increasing activity with temperature up to 50°C. The drop in activity beyond this temperature was less rapid than with toluene-treated cells. The effect on toluene-treated cells of the assay solution pH is shown in Fig. 2; the optimal pH was about 7. Table 2 shows the effect on enzyme activity of various buffers used to prepare the assay solution. Assays performed in sodium phosphate buffer gave the highest enzyme activity, and Tris buffer gave the lowest activity. Some activity was restored if Tris buffer was supplemented with sodium chloride, or, especially, sodium phosphate.

Differential induction rates. Figure 3 shows the induction rate of β-galactosidase of cells grown in a lactose broth. The rate of induction ap-

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<tr>
<th>Table 1. Specific activity of β-galactosidase in untreated whole cells and toluene-treated cells of various organisms*</th>
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<tbody>
<tr>
<td>Culture</td>
</tr>
<tr>
<td>S. lactis 7962</td>
</tr>
<tr>
<td>S. lactis C2</td>
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<tr>
<td>S. lactis C10</td>
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<tr>
<td>S. lactis E</td>
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<tr>
<td>S. lactis 7963</td>
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<tr>
<td>S. lactis 11454</td>
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<td>S. lactis 7902</td>
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* Assays were in sodium phosphate buffer with 0.0004 M MnCl₂·4H₂O at pH 7.0 and 37°C.
proached a constant value of 0.82 units per mg, which was maintained for the remaining period of growth. Figure 4 compares the effect of various compounds on the rate of induction. (The data shown in Fig. 4 were obtained 6 months after the data presented elsewhere in this paper, and show slightly lower differential rates of induction. For example, the rate induced by lactose is 0.72 in Fig. 4a and 0.82 in Fig. 3. This difference in response appears not to be due to a variation in the microorganism. A culture preserved by freezing at the time of the earlier experiments was later compared to a culture maintained by daily transfers throughout this study. The growth and induction characteristics of both these cultures were identical.) Certain nonmetabolizable compounds, such as IPTG and TMG, induce higher rates of enzyme synthesis than lactose in E. coli

<table>
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<tr>
<th>Assay buffer solution</th>
<th>β-Galactosidase specific activity</th>
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<tr>
<td>0.05 M sodium phosphate</td>
<td>0.75</td>
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<tr>
<td>0.05 M potassium phosphate</td>
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<tr>
<td>0.05 M Tris(hydroxymethyl)aminomethane (Tris)</td>
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<td>0.05 M Tris + 0.05 M sodium chloride</td>
<td>0.27</td>
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<tr>
<td>0.05 M Tris + 0.05 M sodium phosphate</td>
<td>0.51</td>
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*All assays were performed at 37°C and pH 7.0 with toluene-treated cells of a 10 hr lactose-induced culture of Streptococcus lactis 7962.

**Fig. 3.** Differential rate of β-galactosidase induction (P) of Streptococcus lactis 7962 grown in lactose broth.

**Fig. 4.** Effect of various compounds on the differential induction rate (P) of β-galactosidase of Streptococcus lactis 7962.
(Cohn and Monod, 1953). However, these compounds induced synthesis of β-galactosidase in S. lactis 7962 less effectively than lactose. Rotman (1964) recently showed that borate plus galactose induced a greater rate of enzyme synthesis in E. coli than either galactose or borate alone; however, as shown in Fig. 4b, this was not true of S. lactis 7962. Borate also caused a 30 to 50% reduction in growth. Melibiose and lactobionate were also poor inducers. The assay substrate, ONPG, induced synthesis of enzyme less effectively than lactose. PNPG was not an inducer. Both ONPG and PNPG, which were hydrolyzed by the cells, retarded growth of the culture.

Repression of enzyme synthesis. Figure 5 demonstrates the effect of exogenously supplied glucose and galactose on β-galactosidase synthesis in lactose-induced cells of S. lactis 7962. Glucose especially repressed enzyme synthesis; galactose only slightly inhibited synthesis.

**DISCUSSION**

During studies on the properties of enzymes in microorganisms, it is generally desirable to measure enzyme activity in presence of substrate under conditions in which the permeability of the cell is not a limiting factor. To attain this, cell-free preparations or toluene-treated cell suspensions have been used. For example, Koppel, Porter, and Crocker (1953) found that toluene-treated cells of E. coli could be used for the assay of β-galactosidase. In the present study, however, work with treated cells was restricted to one strain of S. lactis. This was necessary because it was the only strain among over 40 lactic streptococci tested which produced an enzyme that was stable in either toluene-treated suspensions or cell-free extracts. All attempts to stabilize the enzyme in the other strains with conventional procedures were unsuccessful, but further efforts in this regard are continuing.

The optimal conditions for the assay of the β-galactosidase in S. lactis 7962 were typical of those for other microorganisms in which the enzyme has been studied (Koppel et al., 1953; Anderson and Rickenberg, 1960). Maximal enzyme activity was observed with toluene-treated cell suspensions which were assayed at about 37°C in sodium phosphate buffer of pH 7.0. The requirement for sodium ion to maximally activate the enzyme when ONPG was the substrate was evident; similar results were obtained with E. coli by Cohn and Monod (1951) and with P. aerogenoides by Anderson and Rickenberg (1960).

The ability of certain compounds to induce β-galactosidase in S. lactis 7962 differed from that of other microorganisms. The thiogalactosides TMG and IPTG have been shown to induce enzyme synthesis in E. coli more effectively than lactose, the natural substrate (Cohn and Monod, 1953). It was expected that the same might be true with S. lactis since glucose, an immediate product of the enzyme, was found to repress enzyme synthesis greatly. However, although both TMG and IPTG were inducers of β-galactosidase synthesis in S. lactis 7962, they were not as effective as lactose. In this respect, other microorganisms also have been reported to differ from E. coli. For example, IPTG was an ineffective inducer in S. aureus (McClatchy and Rosenblum, 1963), and TMG was inactive in S. faecium (Buecher and Brock, 1962). Lactose was the only galactoside which induced β-galactosidase synthesis in Paracolobactrum aerogenoides (Anderson and Rickenberg, 1960). However, TMG induced synthesis of about 200 times more enzyme than lactose in Shigella sonnei and Shigella paradyseriae (Rickenberg, 1960). These various responses by bacteria to inducing compounds may indicate permeability differences between organisms or differences in enzyme induction site specificity.

![Fig. 5. Effect of the additions of various carbohydrates on synthesis of β-galactosidase in Streptococcus lactis 7962. Maltose-grown cells were suspended in broth and lactose added at 3 hr. No other additions, curve A; galactose added, curve B; glucose added, curve C; glucose and galactose added, curve D; non-lactose control, curve E. Enzyme assayed in sodium phosphate buffer at pH 7.0 and 37°C.](http://jb.asm.org/)
Induction response to galactose and melibiose also revealed differences between *S. lactis* 7962 and other microorganisms. In the present study, galactose induced synthesis of enzyme nearly 70% as effectively as lactose in *S. lactis*. Galactose, however, was a poor inducer in *E. coli* (Koppel et al., 1953) and *S. sonnei* (Clausen and Nakamura, 1963), but was more active than lactose in *S. aureus* (McClatchy and Rosenblum, 1963). Melibiose, which is an α-galactoside, was almost as effective as lactose in *E. coli* (Koppel et al., 1953) and *S. sonnei* (Rickenberg, 1960), and induced 40 times more enzyme in *S. paradyseriae* (Rickenberg, 1960). Melibiose was neither an inducer of the enzyme in *S. aureus* (McClatchy and Rosenblum, 1963) nor, as found in the present study, in *S. lactis* 7962. Recently, Rotman (1964) reported an increased rate of synthesis of β-galactosidase in *E. coli* by the addition of borate to galactose. This was interpreted to indicate that borate interacted with the transcription mechanism of the lactose operon. This effect could not be demonstrated during the present study with *S. lactis*.

It was found that exogenously supplied galactose severely inhibited β-galactosidase synthesis by lactose-induced cells of *S. lactis* 7962. The rate of enzyme synthesis was therefore regulated not only by lactose, the inducer, but by a metabolite of lactose. Nakada and Magasanik (1964), working with *E. coli*, presented evidence that a product of glucose metabolism, rather than glucose, was actually responsible for the repression of β-galactosidase synthesis. Galactose, the other product of lactose hydrolysis, however, only slightly inhibited enzyme synthesis in *S. lactis*. Results of the present study establish clearly the operation of catabolite repression as a means of control of induced enzyme synthesis in *S. lactis*.

**Acknowledgment**

This investigation was supported by Public Health Service grant EF99 from the National Institutes of Health.

**Literature Cited**


