JOURNAL OF BACTERIOLOGY, June 1980, p. 777-785
0021-9193/80/06-0777/09$02.00/0
Vol. 142, No. 3

Physiological Studies of β-Galactosidase Induction in *Kluyveromyces lactis*

ROBERT C. DICKSON* AND JENNIFER S. MARKIN
Department of Biochemistry, College of Medicine, University of Kentucky, Lexington, Kentucky 40536

We examined the kinetics of β-galactosidase (EC 3.2.1.23) induction in the yeast *Kluyveromyces lactis.* Enzyme activity began to increase 10 to 15 min, about 1/10 of a cell generation, after the addition of inducer and continued to increase linearly for from 7 to 9 cell generations before reaching a maximum, some 125- to 150-fold above the basal level of uninduced cells. Thereafter, as long as logarithmic growth was maintained, enzyme levels remained high, but enzyme levels dropped to a value only 5- to 10-fold above the basal level if cells entered stationary phase. Enzyme induction required the constant presence of inducer, since removal of inducer caused a reduction in enzyme level. Three nongratuitous inducers of β-galactosidase activity, lactose, galactose, and lactobionic acid, were identified. Several inducers of the *lac* operon of *Escherichia coli,* including methyl-, isopropyl- and phenyl-1-thio-β-D-galactoside, and thioallolactose did not induce β-galactosidase in *K. lactis* even though they entered the cell. The maximum rate of enzyme induction was only achieved with lactose concentrations of greater than 1 to 2 mM. The initial differential rate of β-galactosidase appearance after induction was reduced in medium containing glucose, indicating transient carbon catabolite repression. However, glucose did not exclude lactose from *K. lactis,* it did not cause permanent carbon catabolite repression of β-galactosidase synthesis, and it did not prevent lactose utilization. These three results are in direct contrast to those observed for lactose utilization in *E. coli.* Furthermore, these results, along with our observation that *K. lactis* grew slightly faster on lactose than on glucose, indicate that this organism has evolved an efficient system for utilizing lactose.

Kluyveromyces lactis (*Saccharomyces* [24]) is a budding yeast that can use lactose as a carbon and energy source. Lactose is hydrolyzed to glucose and galactose by an intracellular β-galactosidase (EC 3.2.1.23) (6). This organism is interesting because β-galactosidase is induced by lactose and galactose (23), a phenomenon which suggests that these carbohydrates may be regulating expression of the β-galactosidase structural gene. Consequently, we chose to study the mechanism by which lactose induces β-galactosidase in *K. lactis* because of the opportunity to examine the molecular details of gene regulation in a eucaryote. Although this regulatory system might be substantially different from the well-characterized lactose (*lac*) operon of *Escherichia coli,* it enables us to utilize some of the techniques developed for studying the *lac* operon, such as chromogenic substrates for enzyme assays and a variety of substrate analogs for selection of mutants (4). Moreover, we can compare directly the genetic regulatory mechanism of a procaryote and a eucaroyte.

Herman and Halvorson (8) examined the genetics of β-galactosidase synthesis in *K. lactis.* In a cross of two wild-type strains (**a** Y1140 × α Y1118), they found segregation ratios for β-galactosidase activity/nonactivity which suggested that there are two polymeric genes for β-galactosidase, with strain Y1118 being *LAC1 lac2* and strain Y1140 being *lac1 LAC2.* They also (8) demonstrated linkage between *LAC1* and the structural gene for β-glucosidase in strain Y1118, whereas in strain Y1140 no linkage was detected between *LAC2* and the β-glucosidase gene. Other studies have shown that β-galactosidase is induced four- to sixfold in stationary-phase cells by lactose, galactose, and methyl-1-thio-β-D-galactoside (23). Finally, β-galactosidase has been used as an enzyme marker for studies of the cell division cycle. Under noninducing conditions, the basal level of β-galactosidase doubles only during late G2 phase of the cell cycle (20, 27). However, if inducer is added at any phase of the cell cycle, enzyme activity is induced immediately (20).

As part of our effort to determine the molecular mechanism of β-galactosidase induction, we examined in detail the kinetics of enzyme appearance after induction, the types of com-

pounds that induce, and other factors, such as glucose, which might affect the induction mechanism. The results of these studies are presented here.

MATERIALS AND METHODS

Cell strains and culture media. *K. lactis* NRRL a Y1140 (8) was used for all experiments. Cells were maintained on slants of YM medium supplemented with 10 g of glucose and 30 mg of adenine sulfate per liter. Complex YM medium (25) contained the following, per liter: 3 g of malt extract, 5 g of peptone, and 3 g of yeast extract. Yeast nitrogen base medium with amino acids (Difco Laboratories) was prepared according to the manufacturer's directions, except that the concentration was doubled (2× YNB). Another defined medium (DS medium) contained the following, per 100 ml: 2 g of $NH_4H_2PO_4$, 0.167 g of KH_2PO_4, 0.167 g of NaH_2PO_4, 10 μg each of adenine and uracil, 10 mg of $CaCl_2$, 60 mg of $MgSO_4$, 2 mg each of histidine, lysine, and tyrosine, 2 μg of biotin, 400 μg of calcium pantothenate, 2 μg of folic acid, 2 μg of inositol, 400 μg of niacin, 200 μg of *p*-aminobenzoic acid, 400 μg of pyridoxal hydrochloride, 200 μg of riboflavin, 400 μg of thiamine hydrochloride, and 10 μg each of boric acid, copper sulfate, potassium iodide, ferric chloride, sodium molybdate, and zinc sulfate. The pH was adjusted to 5.5 with NaOH. All media were supplemented with a carbon source as indicated below.

Buffers and other reagents. Z buffer (16) contained 0.1 M sodium phosphate, 10 mM KCl, 1 mM $MgSO_4$, and 50 mM 2-mercaptoethanol, pH 7.0. Z-1 buffer had the same composition as Z buffer except that the pH was adjusted to 8.2 with NaOH. All chemicals were reagent grade unless noted otherwise. Methyl-, isopropyl-, and phenyl-1-thio-β-D-galactoside, β-D-galactopyranosyl (1 → 4)-D-gluconic acid (lactobionic acid), and *o*-nitrophenyl-β-D-galactopyranoside were from Sigma Chemical Co. *S*-β-D-galactopyranosyl (1 → 6)-D-glucose (thioallolactose) was a gift from M. Barkely and was originally obtained from W. Boos.

Enzyme assays. β-Galactosidase was assayed spectrophotometrically, with *o*-nitrophenyl-β-D-galactopyranoside as substrate, by the procedure of Miller (16) as modified below. Two procedures were used to assay the enzyme content of intact cells. In procedure A, samples of 100 μl or less were combined with 0.9 ml of Z buffer and enough culture medium to give a total volume of 1.0 ml. This procedure could not be used for larger volumes because the pH of the mixture dropped below 6.9. This problem was particularly acute when samples of different sizes were assayed, as during an induction experiment. To avoid this problem, we developed procedure B, in which the assay mixture contained 0.5 ml of Z-1 buffer, the sample, and enough culture medium to give a final volume of 1.0 ml.

Since *K. lactis* is only slightly permeable to *o*-nitrophenyl-β-D-galactopyranoside, cells were made permeable by blending the assay mixture in a Vortex mixer for 15 s with 3 drops of toluene and then shaking it for 60 to 90 min at 30°C on a gyratory shaker. Thereafter, assay mixtures were equilibrated for 5 min at 30°C, and the reaction was started by adding 0.2 ml of *o*-nitrophenyl-β-D-galactopyranoside (4 mg/ml in water). Reactions were stopped by adding 0.5 ml of 1 M sodium carbonate. Cells were removed by centrifugation, and the absorbance of the supernatant solution at 420 nm (A_{420}) was measured. One unit of enzyme activity equals the production of 1 nmol of *o*-nitrophenol per min per ml. The molar extinction coefficient of *o*-nitrophenol under these conditions is $4.5 \times 10^3 M^{-1} cm^{-1}$ (16).

During an induction experiment, where samples were taken over an extended period of time, it was convenient to store and assay them in batches. This was accomplished by immediately mixing the sample in a Vortex mixer for 15 s with toluene and Z-1 buffer containing 0.04% sodium azide and chilled to 4°C. These samples could be stored at 4°C for up to 5 h without loss of enzyme activity. Thereafter, samples were shaken at 30°C and assayed as described above.

Standard growth procedure. Cells in stationary phase were obtained by growing cells from a slant in 10 ml of medium for 16 to 20 h at 28, 30, or 36°C with continuous aeration. Log-phase cells were obtained by diluting stationary-phase cells into fresh medium to give an A_{600} of 0.15 and growing for 1 cell doubling.

Miscellaneous procedures. Cell densities were measured spectrophotometrically at 600 nm with a Zeiss PM Q2 spectrophotometer. For such measurements, cells were diluted to an A_{600} of less than 0.7; higher densities did not obey the Beer-Lambert function. The number of cells per milliliter was determined by counting cells in a Petroff-Hausser counting chamber. Under our experimental conditions in DS medium, there were 3.8×10^7 log-phase cells per A_{600} unit. Control experiments showed that both cells per milliliter and DNA per milliliter were linear with A_{600} measurements, indicating that the latter measurement was an accurate measure of cell concentration. DNA was measured with diphenylamine (22).

Incorporation of D-glucose-[^{14}C]lactose (Amersham Corp.; CFA 278, 58 mCi/mmol) or [*U*-^{14}C]glucose (New England Nuclear Corp.; NEC-042B, 1 to 5mCi/mmol) into acid-insoluble material was measured by diluting 0.5 ml of cells into 0.5 ml of carrier cells having an A_{600} of 2. Cold 12.5% trichloroacetic acid (4 ml) containing 20 mM lactose and glucose was added immediately, and the sample was stored overnight at 4°C. The sample was centrifuged for 3 min at 4,000 × *g*, and the cell pellet was suspended in 4 ml of cold 10% trichloroacetic acid containing lactose and glucose. Centrifugation and suspension of cells were repeated for a total of four times with no incubation period. Cells were then washed once in 10 ml of 95% ethanol before being suspended in 10 ml of toluene-base scintillation fluid containing 25% (vol/vol) Triton X-100 and 6.5% terphenyl. Samples were counted in a scintillation counter (Packard Instrument Co., Inc.; no. 3003).

RESULTS

Growth rate of *K. lactis*. The growth rate of *K. lactis* Y1140 on various media and carbon sources was measured to determine doubling times and the density at which logarithmic

growth stops. This information was essential for the design of experiments presented in the following sections. Representative growth curves are shown in Fig. 1, and a summary of data is given in Table 1. *K. lactis* grew fastest on lactose, with a doubling time of 91 min in DS medium and 87 min in YM medium. Of the carbon sources tested, cells grew slowest on glycerol, and they only grew well on this compound at temperatures below about 30°C. In contrast, *K. lactis* grew on the other carbon sources listed in Table 1 at temperature as high as 37°C; higher temperatures were not examined. This strain of *K. lactis* could also use fructose, D-mannitol, lactobionic acid, L-sorbose, D-sorbitol, pyruvate, fumarate, succinate (8), and methyl-, isopropyl-, or phenyl-β-D-galactoside as a carbon source (data not shown). In DS medium, citrate, D- or L-fucose, L-arabinose, ethanol, and methyl-, isopropyl-, or phenyl-1-thio-β-D-galactoside did not serve as carbon sources (data not shown).

Rate of β-galactosidase induction as a function of inducer concentration. We determined the rate of increase in β-galactosidase activity, hereafter referred to as the rate of β-galactosidase induction, as a function of inducer concentration to establish the concentration of inducer which gives the maximal rate of induction. Cells were grown in glycerol to avoid the problem of transient carbon catabolite repression (see below). Lactose was used as inducer in all experiments. As shown in Fig. 2, the rate of β-galactosidase induction was maximal with lactose concentrations of greater than 2 mM. Slight induction was observed during the 2-h assay period with 0.125 mM lactose. In another experiment, 1 mM lactose produced the maximal rate of enzyme induction. The data in Fig. 2 were also plotted as differential rates of enzyme induction (units of enzyme per milliliter plotted against culture density, A_{600}) (17) to obviate any differences in growth rates which might exist among the cultures due to the utilization of lactose (data not shown). The differential rate

TABLE 1. *Growth rate of K. lactis Y1140*

Medium	Carbon source[a]	Doubling time[b] (min)
DS	Glycerol	165
DS	Glucose	106
DS	Sorbitol	144
DS	Lactose	91
YM	Glucose	100
YM	Lactose	87
2× YNB	Glucose	123
2× YNB	Lactose	105

[a] The final concentration was 20 mM.

[b] These values represent the average of two or more experiments.

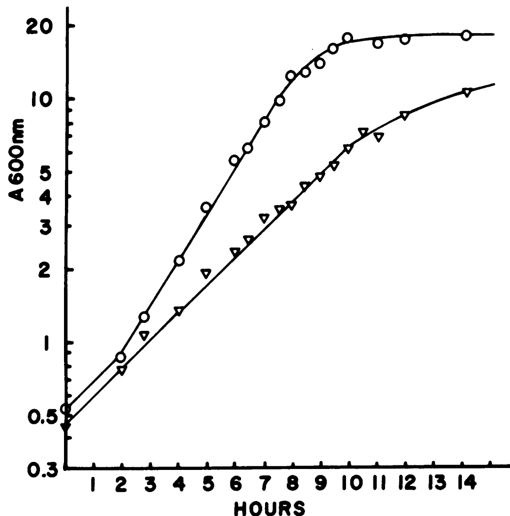

FIG. 1. *Growth curve of K. lactis strain Y1140. Stationary-phase cells were diluted into fresh DS medium containing either 20 mM lactose (○) or sorbitol (▽) as a carbon source and grown at 30°C on a gyratory shaker. Cell density was measured spectrophotometrically at 600 nm.*

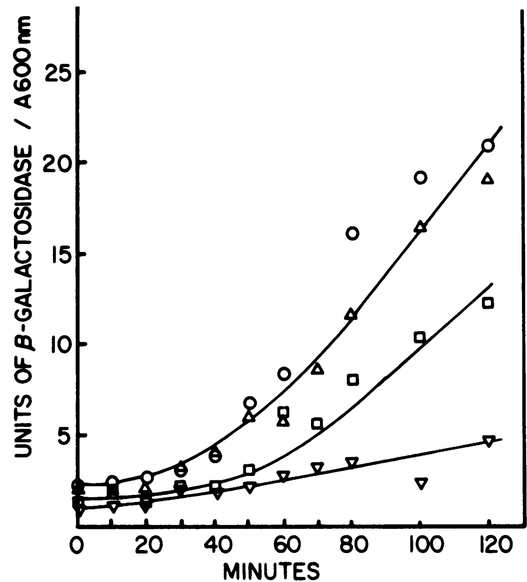

FIG. 2. *Rate of β-galactosidase induction as a function of inducer concentration. Cells were grown to stationary phase in DS medium containing 20 mM glycerol at 28°C, diluted into fresh medium to give an initial A_{600} of 0.15, grown for 1 cell doubling, and then induced at time zero with lactose. Final concentrations (millimolar) of lactose were as follows: ○, 8; △, 2; □, 0.5; and ▽, 0.125.*

of enzyme induction was maximal with lactose concentrations above 1 mM. With 0.5 and 0.125 mM lactose, the differential rate of induction was reduced to ½ and 1/30, respectively. We conclude from these experiments that the maximal rate of β-galactosidase induction is achieved with concentrations of lactose of greater than 1 to 2 mM.

Effect of glucose on β-galactosidase induction. In *E. coli*, the synthesis of β-galactosidase can be influenced by glucose in three ways (14). First, glucose can prevent the entry of lactose into the cell and therefore prevent induction, an effect termed inducer exclusion. Second, the presence of glucose in induced cells reduces the differential rate of β-galactosidase synthesis by half, a phenomenon termed carbon catabolite repression (permanent repression). Third, the initial rate of β-galactosidase synthesis in induced cells is reduced by glucose, a phenomenon termed transient repression. The latter two effects of glucose on β-galactosidase induction result from positive regulation of the *lac* operon by the catabolite gene activator protein and cyclic AMP (reviewed in reference 3). Carbon catabolite repression has also been observed in yeast (see below).

We examined β-galactosidase induction in *K. lactis* to determine whether it was regulated by a glucose-sensitive system. Initially, we found that glucose did not exclude the entry of lactose, since cells grown in glucose could be induced by lactose (see below, Fig. 4b). The two other possible effects of glucose, permanent and transient carbon catabolite repression, were examined by growing cells in DS medium containing either glycerol or glucose as the carbon source and inducing β-galactosidase with lactose. As shown in Fig. 3, cells grown in glycerol and induced by lactose began to synthesize β-galactosidase at the maximal differential rate, whereas cells grown in glucose and induced by lactose did not begin immediately to synthesize β-galactosidase at the maximal differential rate. Instead, there was a short lag period during which the differential rate of enzyme synthesis increased until the maximal rate was reached. Thus, β-galactosidase synthesis in *K. lactis* showed transient, but not permanent, carbon catabolite repression. In this experiment, the differential rate of enzyme synthesis for induced glucose-grown cells was 43 times greater than that for uninduced cells. This value varied from 33 to 100, with an average of 58. Variability was mainly due to inaccuracy in measuring the low enzyme levels in uninduced cells. Cells grown on sorbitol showed almost no transient repression (data not shown).

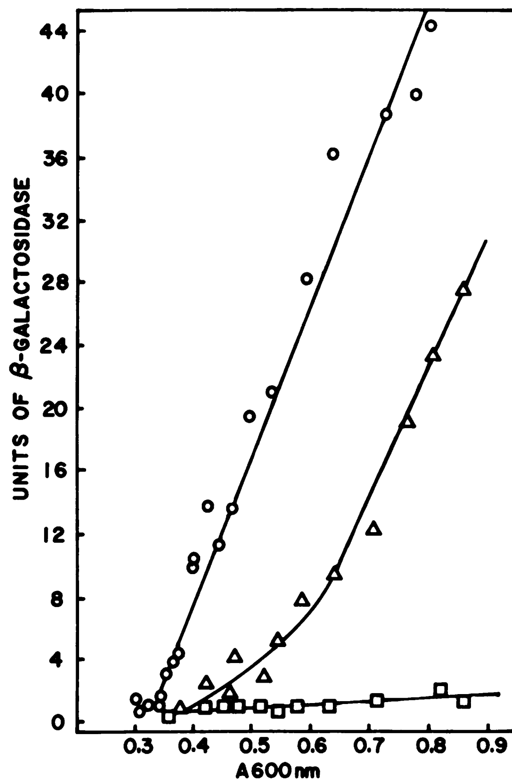

FIG. 3. *Transient repression of β-galactosidase induction. The effect of glucose on the rate of β-galactosidase induction was examined by comparing the differential rate of enzyme induction between cells grown on glucose and those grown on glycerol. Cells were grown to stationary phase in DS medium containing 20 mM glycerol or glucose at 28°C, diluted into fresh medium, allowed to double, and induced with 20 mM lactose at an A_{600} of 0.32 ± 0.02. Cultures were as follows: ○, glycerol-grown plus lactose; △, glucose-grown plus lactose; □, glycerol- or glucose-grown without lactose.*

Effect of glucose on utilization of lactose. The foregoing experiments demonstrate that glucose does not block the induction of β-galactosidase. The experiments do not, however, indicate whether lactose is used as a carbon source after enzyme induction. For example, it is conceivable that lactose transport might be limited by the presence of glucose and that cells would be unable to use lactose even though β-galactosidase is abundant. The data in Fig. 4a (culture C) showed that cells growing in the presence of 20 mM glucose and induced at time zero by the addition of 20 mM [^{14}C]lactose began to incorporate ^{14}C into acid-insoluble material within 60 min. A control experiment demonstrated that incorporation of [^{14}C]lactose required induction since there was no incorporation in an uninduced

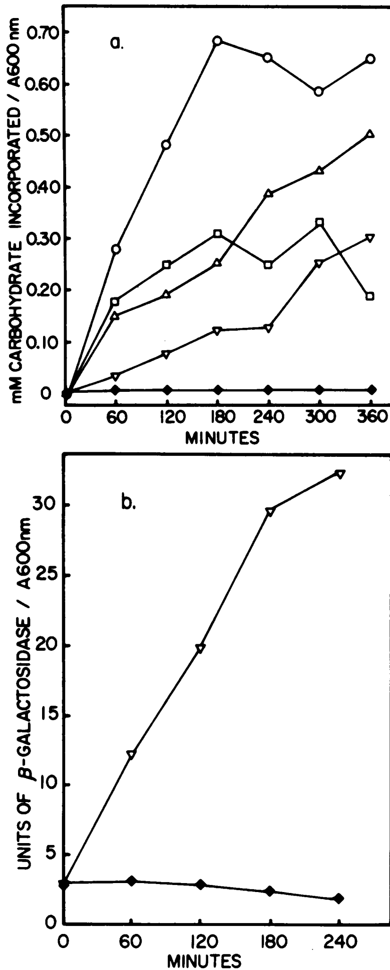

FIG. 4. *Kinetics of lactose utilization in cells growing on glucose. Stationary-phase cells were grown on the carbon source indicated below in DS medium and diluted into 8.5 ml of fresh medium to an* A_{600} *of 0.2. After 2 h of growth at 28°C, the experiment was begun (time zero) by adding 1.5 μCi of [*^{14}*C]lactose or [*^{14}*C]glucose along with a nonradioactive carbohydrate as indicated below. Incorporation of radioactivity into acid-soluble material (a) was measured as described in the text. β-Galactosidase activity (b) was measured by procedure A. Cultures were as follows: A (○), grown on 20 mM glucose, [*^{14}*C]glucose added; B (□), grown on 20 mM glucose, [*^{14}*C]glucose and 20 mM lactose added; C (▽), grown on 20 mM glucose, [*^{14}*C]lactose and 20 mM lactose added; D (△), grown on 20 mM lactose, [*^{14}*C]lactose added; E (◆), grown on 20 mM glucose, [*^{14}*C]lactose added (final concentration of lactose, 6 μM).*

glucose-grown culture containing 6 μM [^{14}C]lactose (culture E, Fig. 4a). This low concentration of lactose did not induce β-galactosidase activity, whereas 20 mM lactose did (Fig. 4b). We conclude that glucose does not prevent either the uptake or the utilization of lactose.

We further examined the utilization of lactose by comparing the rate of [^{14}C]glucose accumulation in uninduced and induced glucose-grown cultures. If lactose is utilized by the induced culture, there should be less accumulation of [^{14}C]glucose. The data shown in Fig. 4a, cultures A and B, verified this prediction and supported our conclusion that lactose can be utilized in the presence of glucose. To determine whether glucose or lactose is preferentially utilized, the amount of glucose incorporated per A_{600} unit was measured after long-term labeling. After 6 and 12 cell doublings, a glucose-grown (20 mM [^{14}C]glucose) culture incorporated twice as many millimoles of glucose as did a glucose-grown culture containing 20 mM lactose. We conclude that there was no preferential utilization of glucose or lactose in a culture containing both carbohydrates.

Kinetics of β-galactosidase induction. Inducible and derepressible enzyme activities in yeast generally increase within a few minutes after induction or derepression. However, this is not true for some enzymes, particularly those involved in long-term adaptation (21, 26) in which several cell generations pass before enzyme activity begins to increase.

The kinetics of β-galactosidase appearance after induction with lactose were examined in *K. lactis* to determine whether enzyme activity appears immediately after the addition of inducer or whether there is a long lag. For these experiments, cells were grown to early log phase in DS medium containing sorbitol and then induced with lactose. Sorbitol was used as a carbon source because it gave the lowest basal level of β-galactosidase (see below, Table 2); cells grew rapidly, and only a slight transient repression was observed. As the insert in Fig. 5 shows, β-galactosidase activity started to increase 10 to 15 min after the addition of inducer at 30°C. Enzyme activity continued to increase for about 7 h (3 to 4 cell doublings) or until a cell density of 5 to 7 A_{600} units per ml was reached; thereafter, enzyme activity decreased (Fig. 5). Although six induction experiments of this type gave similar results, there was variability in the shape of the curve. For example, peak enzyme activities ranged from 50- to 100-fold above the level in an uninduced culture; the peak occurred at a cell density of as low as 4 and as high as 9.5 A_{600} units per ml, and the decrease in enzyme activity occurred at a lower rate. Variation in the induction ratio was due to a twofold day-to-day difference in basal enzyme levels. The reasons for the other variations are unknown.

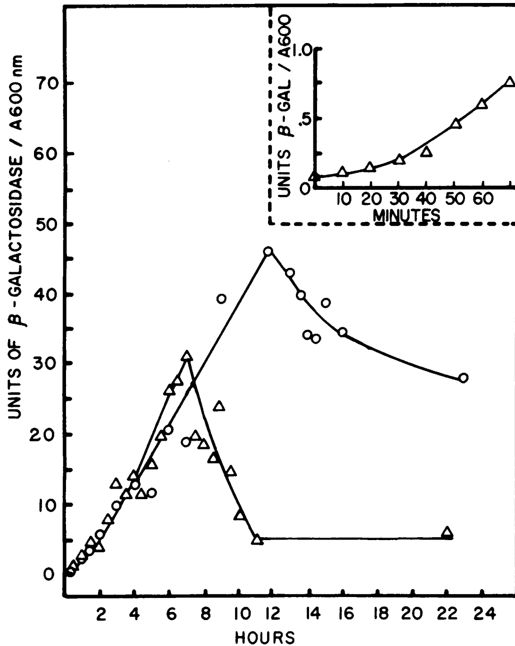

FIG. 5. *Kinetics of β-galactosidase induction. Standard log-phase cells were induced with 20 mM lactose at time zero. Thereafter, cell density and enzyme activity were measured by procedure B as described in the text. In one experiment, cells were allowed to grow to stationary phase (△). In another experiment, cells were kept in log phase by repeated dilution with fresh medium (○). Early time points are shown in the insert. Experimental details are given in the text.*

In the preceding experiment, it appeared as though enzyme activity began to decrease as cells approached stationary phase. Consequently, the maximum or plateau enzyme content per cell may not have been reached. We examined this possibility with cells kept in log phase at an A_{600} of between 0.3 and 0.7 by repeated dilution with fresh medium. Under these conditions, enzyme activity increased for about 12 h (7 to 9 cell doublings) before reaching a maximum value approximately 100-fold higher than the uninduced level (Fig. 5). Again, six experiments of this type gave similar results, but there was variability. For instance, maximum enzyme activity was reached in as short a time as 11 h and as long as 14 h, maximum enzyme levels ranged from a low of 49 to a high of 132 above the uninduced level, and maximum enzyme levels remained constant rather than decreasing 30% as shown in Fig. 5. The reasons for these differences remain unknown.

It is clear from the preceding experiments that maximal β-galactosidase levels per cell are only reached if cells are held in log phase for 11 to 14 h in the presence of inducer (7 to 9 cell doublings); in cells reaching stationary phase before that number of cell doublings, enzyme activity stops short of the maximum level and begins to decrease rapidly.

Inducer withdrawal. In the preceding experiments, β-galactosidase induction was measured on cells exposed continuously to inducer. In these experiments, we examined the response of enzyme levels in induced cells to the withdrawal of inducer. As shown in Fig. 6, β-galactosidase levels stopped increasing and started decreasing within 10 min after inducer was withdrawn. Enzyme levels may start decreasing in less than 10 min, but this would not be determined since 5 to 7 min elapsed between inducer removal and suspension of cells in fresh medium. We concluded from this experiment that enzyme levels increase only in the presence of inducer.

Compounds that induce β-galactosidase. We examined the types of carbohydrates that induce β-galactosidase to characterize further the induction process. Initially, enzyme levels were measured on cells grown for 18 h at 30°C to stationary phase in the presence or absence of inducer. This approach proved unacceptable because: (i) ideally, we wanted to measure maximum induction levels, but as can be seen from the induction curve shown in Fig. 5, measure-

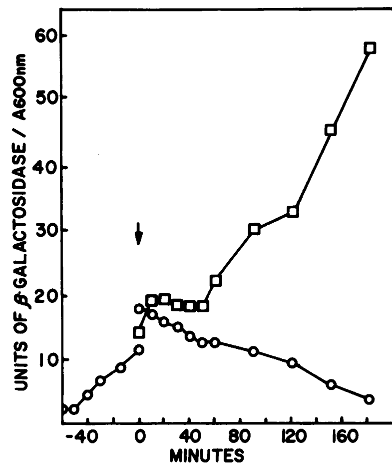

FIG. 6. *Effect of inducer removal on β-galactosidase induction. Log-phase cells growing at 30°C in DS medium containing 20 mM glucose were induced for β-galactosidase by adding lactose to 20 mM at −60 min. At time zero, medium was removed by filtration (0.45-μm membrane filter, type HA, Millipore Corp.), and cells were washed once with 0.5 volume of medium lacking lactose, suspended in fresh medium containing glucose, and divided into two cultures. Lactose was added back to one culture (□), but not to the other (○). β-Galactosidase was assayed by procedure B as described in the text.*

ments made at 18 h did not represent maximum enzyme levels; and (ii) enzyme levels varied up to 10-fold from one experiment to the next for cells grown in the presence of compounds that induce, including lactose, galactose, and lactobionic acid. This variation was due in part to differences in the rate at which enzyme levels decreased after reaching a maximum (Fig. 5). Because of these drawbacks, enzyme levels were measured on log-phase cells grown in the presence of inducer from an A_{600} of 0.5 to an A_{600} of 3 to 5 (about 3 cell doublings). Under these conditions, β-galactosidase levels approached maximum inducible levels as shown in Fig. 5. This procedure proved to be more acceptable because the variability of enzyme levels in induced cultures was reduced and the basal enzyme level in uninduced cultures was reduced two- to fivefold, yielding higher induction values when enzyme levels of induced and uninduced cultures were compared.

Only three of the compounds tested, lactose, galactose, and lactobionic acid, induced β-galactosidase (Table 2). All three compounds gave similar levels of induction, ranging from 116-fold for lactobionic acid to 153-fold for galactose. Isopropyl-1-thio-β-D-galactoside and thioallolactose showed twofold induction values, but this could have been due to contaminating galactose. For example, if 1% of either compound had been hydrolyzed to galactose, then the free galactose concentration would have been 10^{-4} M. As the control experiment in Table 2 shows, 10^{-4} M galactose induced β-galactosidase twofold. We did not examine the purity of the carbohydrates listed in Table 2; therefore, it is possible that those showing low levels of induction could have been contaminated by galactose.

Conceivably, thioallolactose and methyl-, isopropyl-, and phenyl-1-thio-β-D-galactoside, which are good gratuitous inducers of the *E. coli lac* operon (3, 11), failed to induce in *K. lactis* because of exclusion from the cell. This possibility was examined by measuring the intracellular concentration of radioactive methyl- and isopropyl-1-thio-β-D-galactoside. Neither compound was excluded from *K. lactis* since both accumulated to as high an intracellular concentration as lactose (data not shown). Similar experiments with thioallolactose and phenyl-1-thio-β-D-galactoside have not been done.

Although galactose was a good inducer, its 6-deoxy analog, D- and L-fucose, did not induce. *K. lactis* cannot use either of these compounds as a carbon source; therefore, their intracellular accumulation was not examined. The three compounds that induce, lactose, galactose, and lactobionic acid, can be used as carbon sources; therefore, no gratuitous inducer has yet been found.

TABLE 2. *Levels of β-galactosidase elicited by carbohydrates*[a]

Carbohydrate	U of β-galactosidase per A_{600} (avg)	Ratio to sorbitol
Lactose	86.5 ± 6.9	126
Galactose	105 ± 54	153
Lactobionic acid	79.8 ± 17	116
Galactose (10^{-4}M)	1.32 ± 0.46	1.9
IPTG[b]	1.06 ± 0.34	1.5
MTG[b]	0.67 ± 0.21	0.97
φTG[b]	0.82 ± 0.20	1.2
Thiallolactose	1.22 ± 0.38	1.8
D-Fucose	0.83 ± 0.23	1.2
L-Fucose	0.63 ± 0.35	0.91
D-Sorbitol	0.69 ± 0.21	1.0
Glucose	0.90 ± 0.38	1.3

[a] Standard stationary-phase cells were grown at 30°C in DS medium containing 20 mM sorbitol and a 20 mM concentration of the indicated carbohydrate unless noted otherwise. Cells were diluted into fresh medium and grown from 0.5 to 3 to 5 A_{600} units. After treatment with toluene, β-galactosidase was assayed by procedure A as described in the text. Average values represent three or four experiments.

[b] IPTG, Isopropyl-1-thio-β-D-galactoside; MTG, methyl-1-thio-β-D-galactoside; φTG, phenyl-1-thio-β-D-galactoside.

DISCUSSION

Our examination of the kinetics of β-galactosidase induction demonstrated that enzyme activity began increasing 10 to 15 min after the addition of inducer (lactose) at 30°C (Fig. 5). Thus, as with other inducible yeast enzymes (1, 5, 8, 13), the induction of β-galactosidase in *K. lactis* is a rapid process, requiring about 1/10 of a cell division cycle or less. Induction of β-galactosidase is unlike long-term enzyme adaptation (21, 26), in which many cell generations elapse between the addition of inducer and the appearance of enzyme. There are undoubtedly many events which occur during the 10- to 15-min lag after the addition of inducer. Thus far, we have identified one of these events, which involves lactose uptake. Figure 4 shows that the uptake of lactose requires induction. In other experiments, we have shown that the uptake system is indeed inducible by lactose; it requires energy, it is saturable, and it is blocked by genetic mutants, suggesting involvement of a specific permease (R. C. Dickson and K. Barr, unpublished data).

It is clear from our data (Fig. 5) that the maximum inducible β-galactosidase level was only achieved if cells were grown for 7 to 9 cell

doublings in the presence of inducer. If cells entered stationary phase before the necessary number of cell divisions, the maximum β-galactosidase level was not obtained, and the enzyme level decreased. Likewise, the enzyme level decreased shortly after inducer was removed from the culture (Fig. 6). The 80 to 90% decrease in enzyme level which occurred as cells approached stationary phase (Fig. 5) could be explained by dilution due to cell division in the absence of net enzyme synthesis. Alternatively, the enzyme level could decrease because of enzyme inactivation.

Because glucose is a preferred carbon source for numerous organisms, many inducible enzymes cannot be induced when cells are grown in the presence of glucose. This phenomenon, termed carbon catabolite repression, has been analyzed most thoroughly for β-galactosidase induction in *E. coli* (reviewed in reference 14). Similar effects of glucose on inducible enzymes have been noted in yeast. For example, Adams (1) demonstrated that galactokinase induction in *Saccharomyces cerevisiae* was sensitive to both permanent and transient carbon catabolite repression. Recently, Matern and Holzer (15) showed that the addition of glucose to *S. cerevisiae* grown on galactose results in an inactivation of the galactose uptake system. Other glucose-sensitive inducible enzymes in *S. cerevisiae* include maltase and other α-glucosidases (28), invertase (29), alcohol dehydrogenase (5), catalase (19), and enzymes of the citric acid cycle (18).

Our results demonstrated that glucose caused a slight transient repression of the initial differential rate of β-galactosidase synthesis (Fig. 3), but it had no effect on the final differential rate of synthesis, nor did it prevent lactose uptake or utilization (Fig. 4). Thus, there is no permanent carbon catabolite repression of β-galactosidase synthesis or lactose utilization in *K. lactis*. Induction of β-galactosidase activity and lactose utilization in *K. lactis* and *E. coli* clearly respond differently to the presence of glucose. The lack of permanent carbon catabolite repression and inducer exclusion can be explained in two ways; neither explanation excludes the other, and they may both apply. The first explanation is teleological and argues that not only glucose but also lactose is a preferred carbon source because dairy products are the natural habitat for *K. lactis* (24). This possibility was supported by our data showing that *K. lactis* grew faster on lactose than on glucose (Table 1). The faster growth rate on lactose could have been due to faster uptake or to the availability of galactose; either or both possibilities could limit the growth rate on glucose. The second explanation for the absence of permanent carbon catabolite repression and inducer exclusion argues that *K. lactis* lacks such a repression mechanism. In this regard, only one other *K. lactis* enzyme, β-glucosidase, has been examined for carbon catabolite repression; it is atypical because low concentrations (less than 1 mM) of glucose induce, whereas higher concentrations (greater than 10 mM) repress enzyme levels by 40% (9). Since *K. lactis* can use galactose as a carbon source, it would be informative to determine whether the galactose catabolic enzymes are repressible by glucose as they are in *S. cerevisiae* (1, 15).

We examined the types of compounds that induce β-galactosidase to begin characterizing the chemical nature of the natural inducer, to find a gratuitous inducer, and to compare this regulatory system with the *lac* operon of *E. coli*. Two inducers of β-galactosidase in *K. lactis*, lactose and galactose, are also inducers of the *lac* operon (3, 11), whereas lactobionic acid induces *K. lactis* but not the *lac* operon (12). Surprisingly, several good inducers of the *lac* operon (3, 11), including isopropyl-, methyl-, and phenyl-1-thio-β-D-galactoside and thioallolactose, did not induce *K. lactis*. Since isopropyl- and methyl-1-thio-β-D-galactoside accumulate in *K. lactis* cells to as high a concentration as that of lactose, their failure to induce is not due to exclusion from the cell, but could be due to intracellular inactivation. In *E. coli*, isopropyl-1-thio-β-D-galactoside is ultimately inactivated by acetylation (2). We did not examine this possibility directly. However, since *K. lactis* cannot use either of these compounds as a carbon source, it seems unlikely that they are metabolized to any large extent, but minor chemical modifications need to be analyzed. The oxy analog of these two compounds, methyl- and isopropyl-β-D-galactoside, induce β-galactosidase, but this may be due to a release of galactose since *K. lactis* can use either compound as a carbon source. Although these data demonstrate a clear difference between the responsiveness of *K. lactis* and *E. coli* to inducers, it is still possible that the natural inducer of the *lac* operon, allolactose, is also the natural inducer in *K. lactis*. We have shown that purified *K. lactis* β-galactosidase does make allolactose during hydrolysis of lactose (6), as has been noted with *E. coli* β-galactosidase (10). Positive identification of the natural inducer will require experiments with mutants and purified cellular components, as has been done for the *lac* operon (11).

Tingle and Halvorson (23) measured β-galactosidase induction in strain Y1118 of *K. lactis*, a strain related to the strain that we used. They

found that for stationary cells grown in the presence of 6 mM galactose, 3 mM lactose, or 10 mM methyl-1-thio-β-D-galactoside, β-galactosidase was induced 6.4-, 4.6-, and 5-fold, respectively. Their induction values for galactose and lactose are lower than our values (49- and 23-fold, respectively [data not shown]), whereas their value for methyl-1-thio-β-D-galactoside is higher than our value of 2 (Table 2). These differences may be due to strain differences, but this remains to be determined.

These results give an overview of the kinetics of β-galactosidase induction and provide us with a framework for examining the mechanism of induction by biochemical and genetic approaches (7).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM-22749 and Biomedical Research Support grant RR05374-15 from the National Institutes of Health. R.C.D. was supported by Faculty Research Award no. FRA-183 from the American Cancer Society.

LITERATURE CITED

1. **Adams, B. G.** 1972. Induction of galactokinase in *Saccharomyces cerevisiae*: kinetics of induction and glucose effects. J. Bacteriol. **111**:308-315.
2. **Andrews, K. J., and E. C. C. Lin.** 1976. Thiogalactoside transacetylase of the lactose operon as an enzyme for detoxification. J. Bacteriol. **128**:510-513.
3. **Barkley, M. D., and S. Bourgeois.** 1977. The Lac operon, p. 66-127. *In* J. C. Copeland and G. A. Marzluf (ed.), Regulatory biology. Academic Press, Inc., New York.
4. **Beckwith, J. R., and D. Zipser (ed.).** 1970. The lactose operon, p. 8. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
5. **Ciriacy, M.** 1976. Cis-dominant regulatory mutations affecting the formation of glucose-repressible alcohol dehydrogenase (ADH II) in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **145**:327-333.
6. **Dickson, R. C., L. R. Dickson, and J. S. Markin.** 1979. Purification and properties of an inducible β-galactosidase isolated from the yeast *Kluyveromyces lactis*. J. Bacteriol. **137**:51-61.
7. **Dickson, R. C., and J. S. Markin.** 1978. Molecular cloning and expression in *Escherichia coli* of a yeast gene coding for β-galactosidase. Cell **15**:123-130.
8. **Herman, A., and H. Halvorson.** 1963. Identification of the structural gene for β-glucosidase in *Saccharomyces lactis*. J. Bacteriol. **85**:895-900.
9. **Herman, A., and H. Halvorson.** 1963. Genetic control of β-glucosidase synthesis in *Saccharomyces lactis*. J. Bacteriol. **85**:901-910.
10. **Huber, R. E., G. Kurz, and K. Wallenfels.** 1976. A quantitation of the factors which affect the hydrolase and transgalactosylase activities of β-galactosidase (*E. coli*) on lactose. Biochemistry **15**:1994-2001.
11. **Jobe, A., and S. Bourgeois.** 1972. *lac* repressor-operator interaction. VI. The natural inducer of the *lac* operon. J. Mol. Biol. **69**:397-408.
12. **Langridge, J.** 1969. Mutations conferring quantitative and qualitative increases in β-galactosidase activity of *E. coli*. Mol. Gen. Genet. **105**:74-83.
13. **Lawther, R. P., and T. G. Cooper.** 1975. Kinetics of induced and repressed enzyme synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **121**:1064-1073.
14. **Magasanik, B.** 1970. Glucose effects: inducer exclusion and repression, p. 189-219. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Matern, H., and H. Holzer.** 1977. Catabolite inactivation of the galactose uptake system in yeast. J. Biol. Chem. **252**:6399-6402.
16. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Monod, J., A. M. Pappenheimer, Jr., and G. Cohen-Bazire.** 1952. La cinetique de la β-galactosidase chez *E. coli* considerée comme fonction de la croissance. Biochim. Biophys. Acta **9**:648-660.
18. **Polakis, E. S., and W. Bartley.** 1965. Changes in the enzyme activities of *Saccharomyces cerevisiae* during aerobic growth on different carbon sources. Biochem. J. **97**:284-297.
19. **Rytka, J., A. Sledziewski, J. Lukaszkiewicz, and T. Bilinski.** 1978. Haemoprotein formation in yeast. III. The role of carbon catabolite repression in the regulation of catalase A and T formation. Mol. Gen. Genet. **160**:51-58.
20. **Sebastian, J., B. L. A. Carter, and H. O. Halvorson.** 1973. Induction capacity of enzyme synthesis during the cell cycle of *Saccharomyces cerevisiae*. Eur. J. Biochem. **37**:516-522.
21. **Spiegelman, S., R. Sussman, and E. Pinska.** 1950. On the cytoplasmic nature of long-term adaptation in yeast. Proc. Natl. Acad. Sci. U.S.A. **36**:591-606.
22. **Stewart, P. R.** 1975. Analytical methods for yeast. Methods Cell Biol. **12**:111-147.
23. **Tingle, M., and H. O. Halvorson.** 1972. Mutants in *Saccharomyces lactis* controlling both β-glucosidase and β-galactosidase activities. Genet. Res. **19**:27-32.
24. **van der Walt, J. P.** 1970. Genus 8. Kluyveromyces, p. 316-352. *In* J. L. Lodder (ed.), The yeasts. Academic Press, Inc., New York.
25. **Wickerman, L. J.** 1951. Taxonomy of yeasts, p. 1-56. U.S. Department of Agriculture technical bulletin no. 1029. U.S. Department of Agriculture, Washington, D.C.
26. **Winge, O., and C. Roberts.** 1948. Inheritance of enzymatic characters in yeast, and the phenomenon of long-term adaptation. C. R. Trav. Lab. Carlsberg Ser. Physiol. **24**:135-263.
27. **Yashphe, J., and H. O. Halvorson.** 1976. β-D-Galactosidase activity in single yeast-cells during cell cycle of *Saccharomyces lactis*. Science **191**:1283-1284.
28. **Zimmermann, F. K., I. Kaufmann, H. Rasenberger, and P. Haussmann.** 1977. Genetics of carbon catabolite repression in *Saccharomyces cerevisiae*: genes involved in the derepression process. Mol. Gen. Genet. **151**:95-103.
29. **Zimmermann, F. K., and I. Scheel.** 1977. Mutants of *Saccharomyces cerevisiae* resistant to carbon catabolite repression. Mol. Gen. Genet. **154**:75-82.