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Inducible System for the Utilization of β-Glucosides in *Escherichia coli*

I. Active Transport and Utilization of β-Glucosides[1]

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ABSTRACT

Wild-type *Escherichia coli* strains (*β-gl*$^-$) do not split β-glucosides, but inducible mutants (*β-gl*$^+$) can be isolated which do so. This inducible system consists of a β-glucoside permease and an aryl β-glucoside splitting enzyme. Both can be induced by aryl and alkyl β-glucosides. In *β-gl*$^-$ and noninduced *β-gl*$^+$ cells, C^{14}-labeled thioethyl β-glucoside (TEG) is taken up by a constitutive permease, apparently identical with a glucose permease (GP). This permease has a high affinity for α-methyl glucoside and a low affinity for aryl β-glucosides. No accumulation of TEG occurs in a *β-gl*$^-$ strain lacking glucose permease (GP$^-$). In induced *β-gl*$^+$ strains, there appears a second β-glucoside permease with low affinity for α-methyl glucoside and high affinity for aryl β-glucosides. Autoradiography shows that TEG is accumulated by the β-glucoside permease and glucose permease in two different forms (one being identical with TEG, the other probably phosphorylated TEG). In GP$^+$ *β-gl*$^+$ strains with high GP activity, alkyl β-glucosides induce the enzyme and the β-glucoside permease after a prolonged induction lag, and they competitively inhibit the induction by aryl β-glucosides. The induction lag and competition do not exist in GP$^-$ *β-gl*$^+$ strains. It is assumed that phosphorylated alkyl and thioalkyl β-glucosides inhibit the induction, and that this inhibition is responsible for the induction lag.

Escherichia coli strains vary in their capacity to ferment β-glucosides (7, 12). Fermenting and nonfermenting strains have been described. In fermenting strains, the fermentation is due to the presence of fermenting mutants in the nonfermenting wild-type population (18). These mutants ferment salicin and arbutin but not cellobiose. Our previous investigations on several genera of *Enterobacteriaceae* (19–21) have shown that, in the fermentation of β-glucosides, *E. coli* exhibits a substrate specificity different from that of *Escherichia freundii* (*Citrobacter*) and *Salmonella*.

To my knowledge, no data on the enzymology and genetics of the fermentation of β-glucosides by *E. coli* have been published. However, though the uptake of labeled β-glucosides and thioglucosides by *Enterobacteriaceae* has not been previously studied directly, investigations on competition of β-methyl glucoside for the uptake of C^{14}-α-methyl glucoside by a glucose permease of *E. coli* (9) and *S. typhimurium* (11) indicate a high affinity of these permeases for β-methyl glucoside. On the other hand, the glucose permease of *S. typhimurium* has a low affinity for aryl β-glucosides such as phenyl β-glucoside and *p*-nitrophenyl β-glucoside (11).

The wild-type strain of *E. coli* K-12 and several auxotrophic derivatives used in the present study do not ferment β-glucosides, but fermenting mutants were obtained from most of these strains. The present investigations deal with the induction of an aryl β-glucoside splitting enzyme in fermenting mutants, the accumulation of β-glucosides by two permeases, and the relationship between permease activity and induction. The accompanying paper deals with the isolation and characterization of further mutants and with the genetics of the system.

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MATERIALS AND METHODS

Media. The minimal medium used was medium A (5): K_2HPO_4, 7 g; KH_2PO_4, 3 g; $(NH_4)_2SO_4$, 0.1 g; and water, 1,000 ml; supplemented with 0.5% of a carbon source and for solid media with 2% agar. To fulfill growth factor requirements of auxotrophs, amino acids were added at a concentration of 100 μg/ml; purines and pyrimidines, at 40 μg/ml; thiamine, at 10 μg/ml; and vitamin B_{12}, at 0.05 μg/ml. The same minimal medium supplemented with 0.2% yeast extract (Difco), and with 0.5% succinate as carbon source, was used in most induction experiments (medium AY). Acid production and utilization of β-glucosides as carbon source were tested either in solid medium A supplemented with the required growth factors and with 0.5% (w/v) salicin and arbutin, or in the same medium supplemented with 0.075% (w/v) yeast extract, 0.02% (w/v) bromothymol blue, and 0.5% (w/v) salicin (medium ABsal), or 0.5% (w/v) arbutin (medium ABarb).

Chemicals. C^{14}-β-methyl glucoside (specific activity, 1.22 mc/mmole; label in the methyl group) and C^{14}-thioethyl β-glucoside (specific activity, 1.6 mc/mmole; label in the glucose moiety) were purchased from New England Nuclear Corp., Boston, Mass. C^{14}-α-methyl glucoside (specific activity, 1.8 mc/mmole; label in the methyl group) was purchased from Calbiochem. Tritium-labeled salicin was prepared by catalytic exchange by Schwarz Biochemical Co., Orangeburg, N.Y. Thiophenyl β-glucoside samples were gifts from H. O. Halvorson and from M. A. Jermyn. Thioethyl β-glucoside was a gift from J. Duerksen. All other chemicals were purchased from commercial sources.

Strains. The properties and sources of the strains used are listed in Table 1.

Designation of phenotypes. The K-12 wild-type strain and its derivatives listed in Table 1 are of the phenotype β-*gl*$^-$. Cells of this phenotype do not grow on medium A with salicin or arbutin as carbon source; on ABsal and ABarb media, they form small colonies with no color change of the indicator in 36 to 48 hr. Certain K-12 derivatives such as W677, AT12, Hfr (H), and AB1450 produce a weak change of the indicator.

Spontaneous aryl β-glucosides fermenting mutants (phenotype β-*gl*$^+$) were isolated from fermenting papillae on colonies of β-*gl*$^-$ strains grown on medium ABsal or ABarb; β-*gl*$^+$ mutants grow on medium A with salicin or arbutin as carbon source and form large colonies on medium ABsal and ABarb, with change of the indicator. Arbutin-fermenting colonies become black as a result of the oxidation of the liberated hydroquinone. Because of the combination of growth response with indicator change, medium AB can be used for screening of both weak and strong fermenting β-*gl*$^+$ mutants.

Strains having the glucose permease are called "GP$^+$"; this permease is constitutive in the strains employed. The strain W1895 Dl is a glucose permeaseless mutant (GP$^-$) isolated by D. Kessler from the GP$^+$ strain W1895. This strain is unable to phosphorylate α-methyl glucoside (D. Kessler, *personal communication*).

Assay of enzymatic splitting of aryl β-glucosides. One unit of enzyme activity is defined as the amount of enzyme which liberates 1 mμmole of aglycone per min at 35 C and *p*H 7.5. Cells used for determination of enzyme activity were in the logarithmic-growth phase. Further data on media and cell turbidities used are given under Results. The enzyme activity was measured by washing the culture and resuspending it in 0.9 ml of 0.075 M phosphate buffer (*p*H 7.5), containing 10^{-3} M Mg^{++}. The reaction was started by adding 0.1 ml of 2×10^{-2} M substrate and was stoppd (after 10 min if not otherwise specified) by adding 0.5 ml of 2 M Na_2CO_3. The suspension was brought to 3 ml with water; it was then centrifuged, and the liberated aglycone was determined in the supernatant fluid, by using reference curves obtained with known concentrations of the aglycone tested. *p*-Nitrophenol [from *p*-nitrophenyl β-glucoside (1)] and *o*-nitrophenol [from *o*-nitrophenyl β-glucoside (10)] were measured at 410 mμ with a Coleman Junior spectro-

TABLE 1. *List of strains*

Strain[a]	Auxotrophic characters												Fermentation			Sex
	arg	*his*	*ilv*	*leu*	*met*	*pro*	*thr*	*trp*	*thi*	B_{12}	*pur*	*pyr*	*mal*	*mtl*	*xyl*	
K-12 wild type (1)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	F$^+$
Hfr H (2)	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	Hfr
Hfr Cavalli (2)	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	Hfr
AB673 (3)	+	+	+	−	+	+	−	+	−	+	+	+	−	+	+	Hfr
AT1243 (4)	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	Hfr
AT12 (1)	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	Hfr
W1895 (5)	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	Hfr
W1895 Dl (5)	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	Hfr
AB1450 (3)	−	−	−	+	−	+	+	+	−	+	+	+	−	−	−	F$^-$
AB2071 (2)	+	−	−	+	−	−	+	−	−	−	+	+	−	−	+	F$^-$

[a] Numbers in parentheses show origin of strains: (1) W. K. Mass, (2) W. Hayes, (3) E. A. Adelberg, (4) A. T. Taylor, (5) D. Kessler.

photometer. Saligenin (from salicin), phenol (from phenyl β-glucoside), and α-naphthol (from 1-naphthyl β-glucoside) were determined as follows (23). A 1-ml amount of 0.6% 4-aminoantipyrine was added to 2 ml of the alkaline supernatant fluid; after 15 min, 1 ml of 4% $K_3(CN)_6Fe$ was added, and the volume was adjusted to 10 ml with water. The developed color was read after 10 min at 509 mμ. Hydroquinone (liberated from arbutin) was determined by adding 0.5 ml of 10% $NaNO_2$ to 2 ml of the alkaline supernatant fluid. The developed color was read after 60 min at 400 mμ. In induction experiments, the splitting of *p*-nitrophenyl β-glucoside was measured by *p*-nitrophenol determinations in the alkaline supernatant fluid as outlined above. Alternatively, when the presence of the inducer did not interfere with the enzymatic splitting of the substrate (alkyl β-glucosides at all concentrations tested, aryl β-glucosides at concentrations lower than 5×10^{-4} M), 0.1 ml of chloramphenicol (100 μM final concentration) and 0.1 ml of 2.2×10^{-2} M *p*-nitrophenyl β-glucoside were added to 1 ml of culture. The reaction was terminated by adding 0.2 ml of 1 N NaOH, and, after 3 min, 1.6 ml of water. The residual turbidity of the lysed culture was read at 560 mμ and subtracted from the reading at 410 mμ.

Permease activity. To determine permease activity, cells were grown in medium AY, washed with medium A, and resuspended in either medium A or AY at 24 C. The density of cells used for the assay was 400 μg (dry weight) per ml. Chloramphenicol was added to a final concentration of 100 μg/ml, and after 20 min the labeled substrate was added (final radioactivity was 10,000 counts/min). Samples of 1.5 ml were taken at fixed intervals, added to 1.5 ml of chilled medium A, immediately filtered through a 0.45-μ membrane filter (Millipore Filter Corp., Bedford, Mass.), and washed with 4 ml of cold medium A. The filter was air-dried, and radioactivity was counted with a thin-window counter (Nuclear Chicago Corp., Des Plaines, Ill.).

K_m values for the uptake of C^{14}-thioethyl β-glucoside were calculated from Lineweaver-Burk plots of reciprocal values of initial velocities versus concentrations. Initial velocities were determined at 8 C, 2 min after addition of the label. The tested concentration range was 10^{-6} to 5×10^{-4} M. In comparison with 24 C, at 8 C the steady-state concentration is reached with a delay of 8 to 10 min; this permits a more accurate determination of initial velocities.

Accumulated C^{14}-thioethyl β-glucoside was identified by autoradiography. Cells were centrifuged at high speed and the pellet was extracted overnight at 4 C with acetone-ethyl alcohol (4:1). The solvent was evaporated in vacuo, and the residue was dissolved in water. The aqueous solution was then chromatographed by ascending chromatography on Whatman no. 3 paper with butanol-pyridine-water (6:4:3). Kodak X-ray films were exposed for 5 days to the chromatograms and developed. The radioactive spots were cut out and extracted with acetone-ethyl alcohol (4:1). Samples containing equal numbers of counts were evaporated. The residue was dissolved in 0.1 ml of water and mineralized with $Mg(NO_3)_2$-HCl by the procedure of Ames and Dubin (3). Inorganic phosphate was determined by the micromethod of Chen et al. (4).

RESULTS

Enzymatic splitting of β-glucosides. It was found that *β-gl*$^+$ mutants form an inducible aryl β-glucoside-splitting enzyme. No detectable splitting of *p*-nitrophenyl β-glucoside, *o*-nitrophenyl β-glucoside, or other aryl β-glucosides was found with noninduced *β-gl*$^+$ cells, or with most *β-gl*$^-$ strains grown in the presence of inducers. With some *β-gl*$^-$ strains (AB1450, W677, AT1243), splitting of *p*-nitrophenyl β-glucoside was detected after prolonged incubation (2 to 3 hr) after previous induction for four cell generations with 10^{-2} M salicin. The specific activities observed (0.05 to 0.12 units per mg of cell protein) were less than 1% of the activity of their induced *β-gl*$^+$ mutants.

The enzymatic activity of fully induced *β-gl*$^+$ cells varied with the mutant analyzed. Specific activities ranging from 10 to 128 units per mg of cell protein were obtained with *p*-nitrophenyl β-glucoside as substrate. The mutant K-12 *β-gl*$^+$/2 used in most induction and permease experiments showed the following specific activities (units): *o*-nitrophenyl β-glucoside, 180; *p*-nitrophenyl β-glucoside, 102; phenyl β-glucoside, 98; salicin, 82; arbutin, 60; 1-naphthyl β-glucoside, 10.

Sonic treatment (5 min, Raytheon 10-kc sonic oscillator; followed by centrifugation, 20 min, 8,000 rev/min), French pressure cell treatment, and lysis by lysozyme give preparations with only 5 to 9% of the specific activity of whole cells. The enzymatic activity of whole cells was also impaired by toluene and freezing and thawing. This is due to the particulate nature of the enzyme (apparently membrane bound) and cofactor requirements for enzyme activity. Crude sonic extracts of induced K-12 *β-gl*$^+$ cells split *p*-nitrophenyl β-glucoside with a specific activity of 3 to 8 units and with a K_m value of 6.9×10^{-5} M.

Because all the investigated preparations metabolize glucose, the analysis of the fate of the glucose moiety requires further purification of the system.

Enzyme formation. Enzyme biosynthesis was determined in *β-gl*$^+$ cells grown in aerated medium AY plus inducer. The aryl β-glucoside splitting enzyme could also be induced in aerated medium A with succinate or glycerol as carbon source; however, with some *β-gl*$^+$ mutants (AB-1450, AB2071) enzyme formation was lower than in medium AY. The efficiency of the inducers (at saturating concentration) was determined by the differential rate of enzyme synthesis, Δenzyme/Δcell mass (14), and by the length of the induction lag.

Metabolizable inducers. Production of the aryl β-glucoside splitting enzyme could be induced by salicin, *p*-nitrophenyl β-glucoside, *o*-nitrophenyl β-glucoside, phenyl β-glucoside, esculin, and β-methyl glucoside. In most experiments, salicin (concentration range of 10^{-3} to 10^{-2} M), phenyl β-glucoside (concentration range of 10^{-3} to 2.10^{-3} M), and β-methyl glucoside (concentration range of 5.10^{-4} to 10^{-2} M) were used as inducers. Lower concentrations (especially of salicin) were exhausted after two to three cell generations, and the depletion of inducer caused a cessation of enzyme synthesis. Higher concentrations were toxic, causing inhibition of growth and of enzyme biosynthesis.

Gratuitous inducers. Thiophenyl β-glucoside, thioethyl β-glucoside (TEG), and thiomethyl β-glucoside were not metabolizable but they were inducers. The concentration range used was 5×10^{-6} to 2×10^{-3} M for thiophenyl β-glucoside and 2×10^{-5} to 2×10^{-3} M for thiomethyl β-glucoside and TEG. Lower concentrations were ineffective, and higher concentrations were toxic.

β-Methyl xyloside, cellobiose, gentiobiose, amygdalin, α-methyl glucoside, lactose, and thiomethyl β-galactoside were not inducers.

Induction patterns. The analysis of the induction by β-methyl glucoside and salicin in 10 to 15 *β-gl*$^+$ mutants derived from each of 9 different *β-gl*$^-$ strains showed that mutants derived from the same strain are similar in regard to the lag in induction of enzyme biosynthesis. This similarity appears to depend upon the constitutive glucose permease activity of the respective *β-gl*$^-$ parent strains (this property of the parent strains will be discussed further in the next section). Based upon the induction lag with β-methyl glucoside and with salicin, the following induction patterns of *β-gl*$^+$ mutants can be distinguished.

(i) K-12 *β-gl*$^+$ pattern (mutants derived from the K-12 *β-gl*$^-$ wild-type strain): short induction lag (0.5 to 1 cell generations) with salicin or β-methyl glucoside as inducers (Fig. 1A).

(ii) AT12 *β-gl*$^+$ pattern (mutants derived from the *β-gl*$^-$ strains AT12, AB673, AT1243): short induction lag with salicin, induction lag of two to three cell generations with β-methyl glucoside or salicin plus β-methyl glucoside (Fig. 1B). (Higher ratios of salicin/β-methyl glucoside gave intermediate values.)

(iii) Hfr Cavalli pattern [mutants derived from the *β-gl*$^-$ strains Hfr Cavalli and Hfr(H)]: induction lag of two to three cell generations with salicin, induction lag of four to five cell generations with β-methyl glucoside or salicin plus β-methyl glucoside (Fig. 1C).

The induction curves of the *β-gl*$^+$ mutants of the *β-gl*$^-$ strains AB2071 and W1895 were characterized by an induction lag intermediate between that of the AT12 and Hfr Cavalli mutants, and the induction lag observed with W677 *β-gl*$^+$ mutants was intermediate between AT12 and K-12 *β-gl*$^+$ mutants.

Phenyl β-glucoside and thiophenyl β-glucoside behaved like salicin; the induction due to TEG

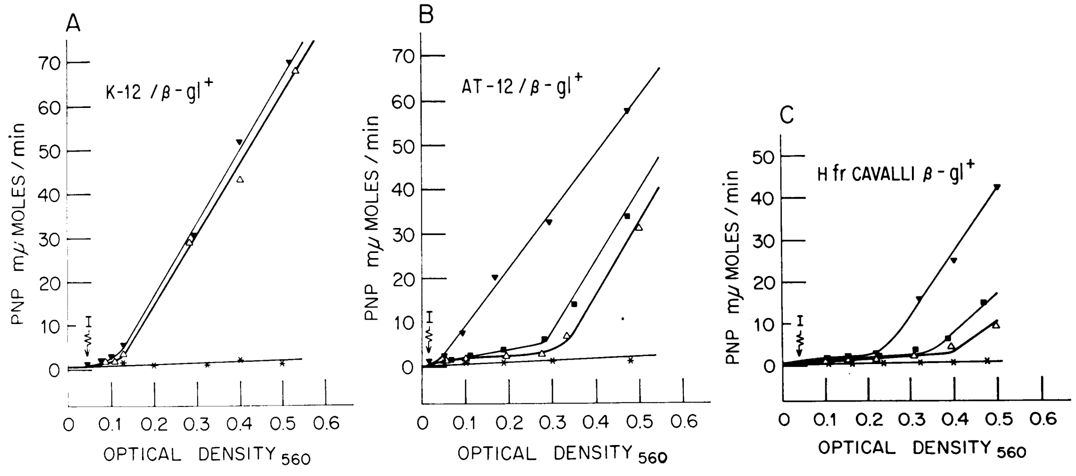

FIG. 1. *Induction patterns. Comparison of the induction patterns of the β-gl*$^+$ *mutants of β-gl*$^-$ *strains. Cultures were grown in aerated medium AY at 35 C. Samples for enzyme activity were washed in 0.075* M *phosphate buffer (pH 7.5) and incubated for 10 min with* 2×10^{-3} M *p-nitrophenyl β-glucoside. The optical density of cultures was determined at 560 mμ. (A) Mutant K-12 β-gl*$^+$*/2 of the strain K-12 wild type. (B) Mutant AT12 β-gl*$^+$*/5 of the strain AT12. (C) Mutant Hfr Cavalli β-gl*$^+$*/2 of the strain Hfr Cavalli.* ▼ = *induced with salicin* (10^{-2} M); △ = *induced with β-methyl glucoside* (10^{-2} M); ■ = *induced with salicin* (10^{-2} M) + *β-methyl glucoside* (10^{-2} M); * = *no inducer added.*

and thiomethyl glucoside was like that by β-methyl glucoside.

Active uptake of β-glucosides. Experiments with C^{14}-TEG showed that TEG was taken up by two different permeases. One (permease I) was constitutive. It could be found in β-gl^- and β-gl^+ cells, and its characteristics were similar to those described in the literature for the glucose permease. The second (permease II) was inducible and could be found only in induced β-gl^+ cells. Permease I will be referred to as "glucose permease" and permease II as "β-glucoside-permease."

Uptake of TEG by the glucose permease. Pertinent published data on glucose permease show that both glucose and α-methyl glucoside are substrates of the glucose permease, which also has a high affinity for β-methyl glucoside and a low affinity for aryl β-glucosides (11). The steady-state value of the intracellular concentration of α-methyl glucoside is the result of an active entry reaction and of an active exit reaction. The exit reaction is more sensitive to deprivation of energy source than the entry reaction, and therefore the absence of a metabolizable carbon source or the addition of 2,4-dinitrophenol in the presence of a carbon source will increase the steady-state concentration by blocking the exit reaction (8). It was also reported that α-methyl glucoside is phosphorylated by *E. coli* cells (9), and at low external concentration phosphorylation is essential for its accumulation (D. Kessler, *personal communication*).

The uptake of C^{14}-TEG by K-12 β-gl^- wild-type cells and noninduced K-12 β-gl^+ cells, possessing permease I and lacking permease II, showed characteristics similar to those of the uptake of α-methyl glucoside. These similarities included a high affinity for glucose and α-methyl glucoside and a low affinity for the aryl β-glucosides phenyl β-glucoside and salicin, as judged by inhibition of TEG uptake (Table 2). The steady-state concentration of TEG was higher in medium A without carbon source than in medium AY with succinate as principal metabolizable carbon source (Table 2, Fig. 2A). In medium AY, 2,4-dinitrophenol (5×10^{-3} M) increased the steady-state value of the accumulation of C^{14}-TEG by β-gl^- cells from 0.41 to 3.61 μmoles/g and from 0.88 to 4.28 μmoles/g by noninduced K-12 β-gl^+ cells.

The K_m value for the uptake of TEG by noninduced K-12 β-gl^+ cells was 1.5×10^{-4} M in medium A and 0.84×10^{-4} M in medium AY.

Further indications for the identity of permease I with a glucose permease were obtained by uptake experiments with glucose permease-negative cells. It was found that, at an external concentration of 10^{-4} M, strain W1895 GP^+ β-gl^- accumulated, at steady state in medium A, 18.2 μmoles/g of C^{14}-α-methyl glucoside and 7.3 μmoles/g of C^{14}-TEG (45- and 18-fold concentration from the medium); the glucose permease-negative mutant 1895 Dl GP^- β-gl^- accumulated 0.39 μmole/g of α-methyl glucoside and 0.36 μmole/g of TEG, showing no concentration of either compound from the medium.

Uptake of TEG by the "β-glucoside permease." The determination of TEG uptake by the β-glucoside permease was complicated by the fact that induced β-gl^+ cells took up TEG with both permeases. However, noninduced K-12 β-gl^+ cells had a relatively low glucose permease activity (Table 3), and in fully induced K-12 β-gl^+ cells

TABLE 2. *Uptake of C^{14}-TEG by β-gl^- and β-gl^+ cells*[a]

Addition	K-12 β-gl^+ (TM glu)		K-12 β-gl$^+$ (noninduced)		K-12 β-gl^- (TM glu)		K-12 β-gl^- (noninduced)	
	AY	A	AY	A	AY	A	AY	A
TEG	100 (6.90)[b]	100 (2.80)	100 (0.95)	100 (2.68)	100 (0.61)	100 (1.62)	100 (0.75)	100 (3.28)
TEG + α-methyl glucoside	98	72	31	19	28	13	23	22
TEG + glucose	138	108	23	9	8	5	18	8
TEG + phenyl β-glucoside	3	7	—	76	73	78	72	94
TEG + salicin	7	20	134	97	68	104	94	138

[a] Cells of the strain K-12 β-gl^- and of its β-gl^+ mutant K-12 β-$gl^+/2$ were grown from an optical density of 0.04 to an optical density of 0.35 in medium AY (noninduced) and medium AY with 2×10^{-4} M thiomethyl β-glucoside (TM glu). The uptake of TEG (10^{-4} M external concentration) was determined in media A and AY. α-Methyl glucoside, glucose, phenyl β-glucoside, and salicin were added to a final concentration of 10^{-3} M. The uptake of TEG (micromoles per gram) was determined 10 min after the addition of the compound and is expressed as the percentage of uptake by the same cell suspension without addition.

[b] Uptake of TEG in micromoles per gram without addition.

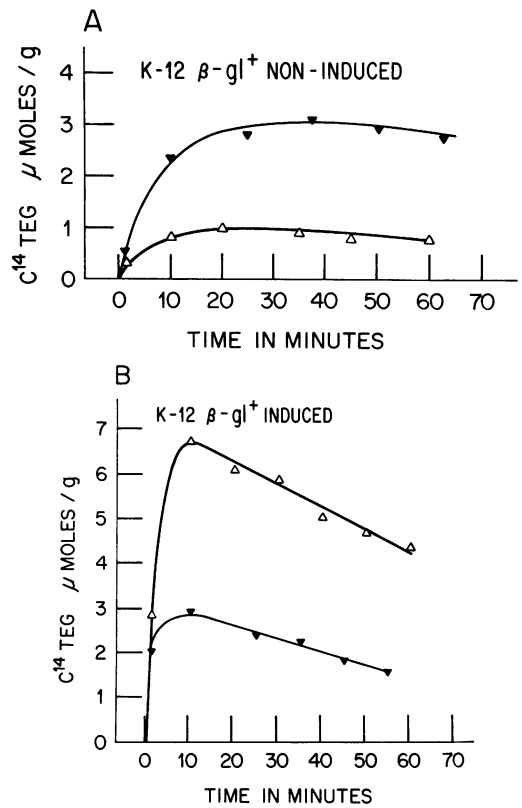

FIG. 2. *Uptake of TEG in media A and AY. Cultures of the K-12 β-gl^+/2 mutant were grown at 35 C from an optical density of 0.4 to an optical density of 0.35 in medium AY (noninduced) and medium AY with 2×10^{-4} M thiomethyl β-glucoside (induced). The cultures were washed in medium A and resuspended in media A and AY with 100 μg/ml of chloramphenicol. After 20 min at 24 C, TEG (final concentration, 10^{-4} M; 10,000 counts/min) was added. (A) = K-12 β-gl^+ noninduced cells. (B) = K-12 β-gl^+ induced cells. Symbols: △ = uptake in medium AY; ▼ = uptake in medium A.*

the uptake of TEG occurred mainly through the β-glucoside permease. The uptake by fully induced K-12 β-gl^+ cells showed the following characteristics. Judged by inhibition studies, the β-glucoside permease had a low affinity for glucose and α-methyl glucoside and a high affinity for aryl β-glucosides (Table 2). In contrast to β-gl^- and noninduced β-gl^+ cells, the uptake of TEG was higher in medium AY, in the presence of a carbon source (Fig. 2, Table 2), and 2,4-dinitrophenol did not increase the steady-state concentration of TEG. In medium A, although the internal concentration of TEG was similar in induced and noninduced β-gl^+ cells, the inhibition data with α-methyl glucoside and aryl β-glucosides indicated that TEG was taken up in induced cells mainly by the β-glucoside permease. The K_m value for TEG uptake by induced K-12 β-gl^+ cells in medium A was 1.9×10^{-5} M and 0.75×10^{-5} M in medium AY, approximately 10 times lower than that found with noninduced cells.

The data obtained with induced K-12 β-gl^+ cells, in which both permeases are active, were compared with the uptake by the β-glucoside permease alone by using a β-gl^+ mutant of the glucose permease-negative strain W1895 Dl or by using tritium-labeled salicin as substrate, because glucose permease has a low affinity for salicin. At steady-state concentration in medium AY, induced W1895 Dl GP$^-$ β-gl^+ cells accumulated 6.9 μmoles/g of TEG. The K_m value for the uptake of TEG was 0.67×10^{-5} M, and inhibition experiments indicated an affinity for salicin and phenyl β-glucoside similar to that found with induced K-12 β-gl^+ cells. The uptake of tritium-labeled salicin in medium A was determined with the strains K-12 β-gl^- and K-12 β-gl^+ sal^- c_1 (22). The latter strain lacked the ability to split salicin and was constitutive for splitting of p-nitrophenyl β-glucoside and the β-glucoside permease. Its isolation will be described in the following paper. It was found that β-gl^- cells, which possess only the glucose permease, did not accumulate salicin. In the cells of the strain K-12 β-gl^+ sal^- c_1, which presumably accumulate salicin only through the β-glucoside permease, the internal concentration of salicin reached 3.2×10^{-2} M (external concentration, 10^{-4} M), much higher than the maximal accumulation of TEG by the same strain (2.1×10^{-3} M). This is consistent with the high affinity of the β-glucoside permease for aryl β-glucosides.

Autoradiography. Autoradiography for C^{14} of acetone-ethyl alcohol extracts from cells of strain W1895 Dl GP$^-$ β-gl^+ c_1 (22), which lacks glucose permease and is constitutive for the β-glucoside permease, and of strain W1895 GP$^+$ β-gl^-, which possesses only the glucose permease, showed the following: GP$^-$ β-gl^+ c_1 cells accumulated C^{14} TEG in an unchanged form, with R_F of 0.66, identical with that of the original compound, whereas GP$^+$ β-gl^- cells accumulated TEG mainly in a form with R_F 0.79, different from that of the original compound. Preliminary studies on the nature of the compound accumulated by the glucose permease showed that the compound with R_F 0.79 contained 1.08 μmoles of P per 12,000 counts, whereas at an equal number of counts the compound with R_F 0.66 contained only 0.085 μmole of P. This is consistent with the published data (9) on accumulation of α-methyl glucoside by the glucose permease in a phosphorylated form.

Induction pattern and glucose permease activity. The uptake of C^{14}-TEG, C^{14}-α-methyl glucoside, and C^{14}-β-methyl glucoside was determined with noninduced cells of the strain K-12 β-*gl*$^+$ (short induction lag when induced by alkyl β-glucosides) and of the strains AT12 β-*gl*$^+$, Hfr Cavalli β-*gl*$^+$, and AB2071 β-*gl*$^+$ (with prolonged induction lag). The strains with prolonged induction lag had a higher glucose permease activity (Table 3), thus suggesting a possible relationship between the induction pattern of these mutants (Fig. 1) and their glucose permease activity.

TABLE 3. *Uptake of C^{14}-α-methyl glucoside and C^{14}-alkyl β-glucosides by noninduced β-gl$^+$ cells*

Compound	Uptake (µmoles/g)[a]			
	K-12	AT12	Hfr Cavalli	AB2071
TEG, 10^{-4} M	3.10	6.78	7.28	6.59
α-Methyl glucoside, 10^{-5} M	0.73	5.78	—	—
α-Methyl glucoside 10^{-4} M	10.50	17.60	19.52	17.94
β-Methyl glucoside 10^{-4} M	1.50	5.60	—	—

[a] Maximal uptake in medium A at 24 C.

To test this hypothesis, the β-*gl*$^+$ allele from strain AT12 β-*gl*$^+$ was transduced with Plkc phage into strain W1895 GP$^+$ with high glucose permease activity and into its GP$^-$ mutant W1895 Dl. The resulting strains were grown in medium AY, medium AY with 10^{-4} M thiomethyl β-glucoside, medium AY with 10^{-4} M thiophenyl β-glucoside, and an equimolar mixture of the two (Fig. 3A, B).

Thiomethyl β-glucoside produced a prolonged induction lag with GP$^+$ β-*gl*$^+$ cells and inhibited competitively the induction by thiophenyl β-glucoside (Fig. 3A), an effect not observed with GP$^-$ β-*gl*$^+$ cells. This difference in the induction pattern of GP$^+$ β-*gl*$^+$ and GP$^-$ β-*gl*$^+$ cells indicates that thiomethyl β-glucoside inhibited the induction by thiophenyl β-glucoside only when the glucose permease was present.

Inhibition of induction of β-galactosidase. In *E. coli* strains, α-methyl glucoside inhibited the induction of β-galactosidase (13, 24). This inhibition did not occur in the GP$^-$ strain (D. Kessler, *personal communication*). In the present study, it was found that β-glucosides and β-thioglucosides

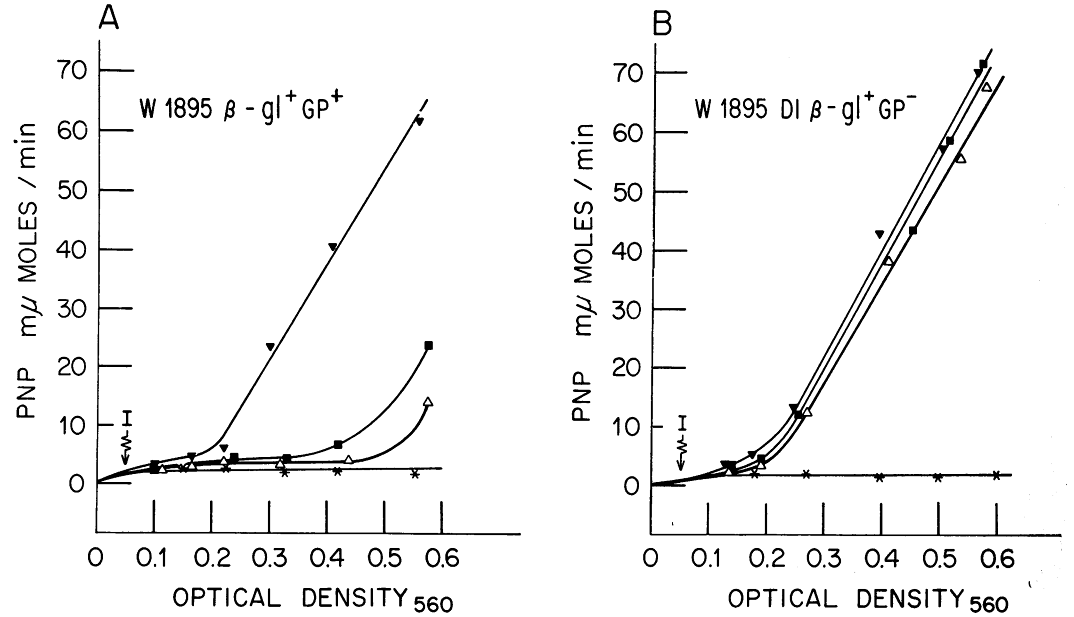

FIG. 3. *Induction of GP$^+$ β-gl$^+$ and GP$^-$ β-gl$^+$ strains. Induction by thiomethyl β-glucoside (10^{-4} M), thiophenyl β-glucoside (10^{-4} M), and their equimolar mixture. Cultures were grown in aerated medium AY at 35 C. To 1 ml of culture, p-nitrophenyl β-glucoside (final concentration, 2×10^{-3} M) and chloramphenicol (final concentration, 100 µg/ml) were added. Incubation was for 10 min. Optical density of cultures was determined at 560 mµ. (A) Strain W1895 GP$^+$ β-gl$^+$. (B) Strain W1895 Dl GP$^-$ β-gl$^+$. Symbols: ▼ = thiophenyl β-glucoside (10^{-4} M); △ = thiomethyl β-glucoside (10^{-4} M); ■ = thiophenyl β-glucoside (10^{-4} M) + thiomethyl β-glucoside (10^{-4} M); * = control.*

also inhibited the induction of β-galactosidase. This fact permitted the investigation of the influence of the uptake of β-glucosides by both permeases upon the induction of β-galactosidase. The strains used were W1895 GP$^+$ *β-gl*$^-$ (possessing only glucose permease), W1895 Dl GP$^-$ *β-gl*$^-$ (lacking both permeases), and W1895 Dl GP$^-$ *β-gl*$^+$ c_1 (lacking glucose permease and constitutive for the β-glucoside permease). The concentrations of 5 × 10^{-4} M for the inhibitor and of 10^{-4} M for the inducer (thiomethyl β-galactoside) were found to be optimal. The results are shown in Fig. 4.

As expected with the W1895 GP$^+$ *β-gl*$^-$ strain, α-methyl glucoside, β-methyl glucoside, and especially thiomethyl β-glucoside inhibited the induction of β-galactosidase. This inhibition was indicated mainly by the length of the induction lag rather than by the slope of the curve. Thiophenyl β-glucoside had no effect, presumably due to the absence of the β-glucoside permease (Fig. 4A). With strain W1895 Dl GP$^-$ *β-gl*$^-$, lacking both permeases, thiomethyl β-glucoside was a weak inhibitor (Fig. 4B). With strain W1895 GP$^-$ *β-gl*$^+$ c_1, α-methyl glucoside had no effect, owing to the absence of the glucose permease, whereas both thiomethyl and thiophenyl β-glucoside were inhibitors (Fig. 4C). Experiments with different concentrations of thiomethyl β-glucoside showed that the inhibition of the induction of β-galactosidase by thiomethyl β-glucoside taken up by each permease was competitive. These data are consistent with the view that alkyl β-glucosides and thioglucosides can be taken up by both permeases, whereas aryl β-glucosides and thioglucosides can be taken up only by the β-glucoside permease. The inhibition of the induction of β-galactosidase seems to be independent of the permease by which the uptake occurs.

DISCUSSION

The mutation from *β-gl*$^-$ to *β-gl*$^+$ in *E. coli* K-12 strains was characterized by the appearance of an inducible permease and of an aryl β-glucoside splitting enzyme (or enzymes). It was found that aryl β-glucosides are better inducers than alkyl β-glucosides. This situation appears to be different from that found in *Rhodotorula minute* (6), where aryl β-glucosides are poor inducers for β-glucosidase.

The appearance of the *β-gl*$^+$ phenotype could be the result of a mutation in the structural gene of the β-glucoside permease or of a mutation from noninducibility to inducibility in a regulatory gene. Attempts to force enzyme biosynthesis in *β-gl*$^-$ cells by increasing the concentration of inducers and by growth in the presence of inducers and dimethylsulfoxide or ethylenediaminetetraacetate were unsuccessful (S. Schaefler, *unpublished data*). Therefore, a clear-cut differentiation between these two hypotheses is not yet possible.

An important feature of the system is that the

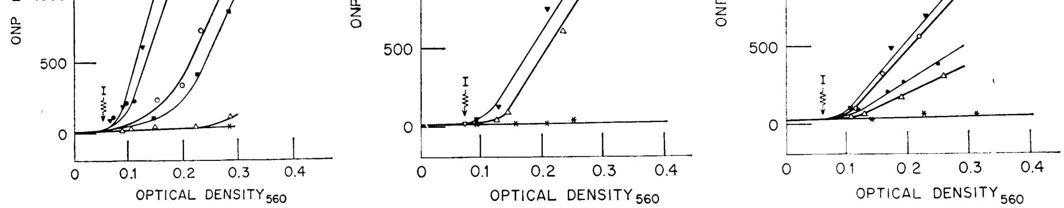

FIG. 4. *Cultures grown in medium AY at 35 C. Induction by thiomethyl β-galactoside* (10^{-4} M). *α-Methyl glucoside, β-methyl glucoside, thiomethyl β-glucoside, and thiophenyl β-glucoside were added to a final concentration of 5* × 10^{-4} M. *To 1 ml of toluene-treated culture, suitably diluted in AY medium, 0.5 ml of 2.5* × 10^{-2} M *o-nitrophenyl β-galactoside in 0.075* M *phosphate buffer (pH 7.0) was added, followed after 5 min by the addition of 0.5 ml of 2* M Na_2CO_3. *The developed color was read at 410 mμ. (A) W1895 GP*$^+$ *β-gl*$^-$. *(B) W1895 Dl GP*$^-$ *β-gl*$^-$. *(C) W1895 Dl GP*$^-$ *β-gl*$^+$ c_1. *Symbols:* ▼ = *thiomethyl β-glucoside (TMG);* ■ = *TMG + β-methyl glucoside;* ○ = *TMG + α-methyl glucoside;* △ = *TMG + thiomethyl β-glucoside;* ● = *TMG + thiophenyl β-glucoside;* * = *noninduced.*

same compound is taken up by two permeases. Such a situation has been described in other systems also, e.g., in the accumulation of β-galactosides (17), melibiose (16), and aromatic amino acids (2). Data on the physiological role of multiple permeases are still scarce. The following hypothesis is proposed for the uptake of β-glucosides and thioglucosides. When taken up by the β-glucoside permease, β-glucosides and thioglucosides are accumulated in a nonmodified form called β-I; in this form they are inducers. When taken up by the glucose permease, they are accumulated in a modified form (probably a phosphorylated derivative) called β-NI. The β-NI form is not an inducer; it competitively inhibits the induction by the β-I form. Alkyl β-glucosides are accumulated in the β-NI form by the glucose permease and in the β-I form by the β-glucoside permease; aryl β-glucosides are accumulated only in the β-I form. Both the β-I and the β-NI form of β-glucosides are competitive inhibitors of the induction of β-galactosidase. This competitive inhibition of β-galactosidase, which is similar to that obtained with some other compounds such as nitrophenyl β-fucoside (15), appears to be qualitatively different from catabolite repression.

Considering the *β-gl⁻* → *β-gl⁺* mutation as a change in the β-glucoside permease, one can explain the inability of *β-gl⁻* strains to form the aryl β-glucoside splitting enzyme by the accumulation of alkyl β-glucosides in the β-NI form by the glucose permease and the lack of accumulation of aryl β-glucosides by the lack of the β-glucoside permease. A high degree of impermeability of the cell membrane to the diffusion of β-glucosides prevents their entry by diffusion even at very high external concentrations. If one considers the *β-gl⁻* → *β-gl⁺* mutation as a regulatory mutation, the lack of induction of *β-gl⁻* cells can be explained by the effect of noninducibility and the absence of the β-I form of β-glucosides.

In *β-gl⁺* mutants derived from *β-gl⁻* strains with high glucose permease activity (e.g., AT12 *β-gl⁺*), an alkyl β-glucoside, such as methyl β-glucoside, presumably reaches a high internal concentration of its β-NI form very fast, due to the accumulation by the glucose permease. The high concentration of the β-NI form inhibits competitively the enzyme induction by the β-I form of the same compound. The result is an induction lag. The competitive inhibition by alkyl β-glucosides of the induction by aryl β-glucosides occurs in the same way. The presumed mode of action in *β-gl⁺* cells of the β-NI and β-I form of β-glucosides is independent of whether the *β-gl⁺* mutants appear as a result of a permease or regulator mutation.

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