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Genetic Determination of the Constitutive Biosynthesis of Phospho-β-Glucosidase A in *Escherichia coli* K-12

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Escherichia coli wild-type cells form constitutively the enzyme phospho-β-glucosidase A, which has a high affinity for phosphorylated aromatic β-glucosides and a low affinity for phosphorylated β-methyl-glucoside. Phospho-β-glucosidase B and β-glucoside permease I are formed in aromatic β-glucoside-fermenting mutants. Mutants lacking phospho-β-glucosidases A and B have been isolated. These mutants showed a reduced rate of inducibility of the β-glucoside permease I. The restoration of phospho-β-glucosidase A or B activity resulted in an increased rate of induction of the β-glucoside permease I. The presence of the phospho-β-glucosidases was not required for the constitutive biosynthesis of the β-glucoside permease. Mutants selected for growth on β-methyl-glucoside as carbon source showed an increased level of constitutive phospho-β-glucosidase A activity. Gene *bglD*, the structural gene for phospho-β-glucosidase A, was mapped between the *pyrE* locus and the cluster *bgl* loci, whereas *bglE*, the regulatory site determining the hyperproduction of phospho-β-glucosidase A, was mapped between the *bgl* and *ilv* clusters. The *bglE* locus appears to have a regulatory effect on the expression of the *bglD* gene.

In *Escherichia coli* K-12, β-glucosides are accumulated in a 6-phosphorylated form by two species of enzyme II that were designated as β-glucoside permease I and glucose permease (1, 4, 7). The glucose permease is constitutive and accumulates only aliphatic β-glucosides; β-glucoside permease I is inducible and accumulates aromatic and aliphatic β-glucosides (4, 7). Phosphorylated β-glucosides are hydrolyzed into glucose-6-phosphate (G-6-*P*) and aglycone by phospho-β-glucosidase A and B (*P*-β-glucosidase A and B, EC 3.2.1.21; 1, 7). *P*-β-glucosidase A hydrolyzes phosphorylated *p*-nitrophenyl-β-glucoside (PNP-glu), phenyl β-glucoside, and arbutin, but not salicin. *P*-β-glucosidase B, which is more thermolabile, hydrolyzes phosphorylated salicin and the same substrates as *P*-β-glucosidase A. Both enzymes have a low affinity for phosphorylated β-methyl-glucoside (G. Wilson and F. Fox, in preparation). Wild-type cells possess constitutive glucose permease and *P*-β-glucosidase A activities, but lack β-glucoside permease I and *P*-β-glucosidase B. Because of the lack of β-glucoside permease I, intact cells, although they possess the constitutive *P*-β-glucosidase A, are unable to catabolize aromatic β-glucosides such as PNP-glu and arbutin. Wild-type cells show weak utilization of β-methyl-glucoside which can be accumulated through the glucose permease. A spontaneous mutation at the regulatory site, *bglB* (5; Prasad and Schaefler, unpublished data) allows the expression of inducible biosynthesis of β-glucoside permease I and *P*-β-glucosidase B. This enables the cells to utilize aromatic β-glucosides which are accumulated by β-glucoside permease I.

The structural gene for *P*-β-glucosidase B (*bglA*) and the regulatory sites *bglB* and *bglC* have been previously mapped at 73 min of the *E. coli* linkage map (5, 8). The present paper deals with the isolation and genetic analysis of two types of mutants affecting the activity of *P*-β-glucosidase A. The first type lacks *P*-β-glucosidase A activity because of a mutation in the *bglD* locus, which is probably the structural gene for the enzyme. The second type has an increased level of constitutive *P*-β-glucosidase A

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activity because of a mutation at the *bglE* locus, which is apparently a regulatory gene for this enzyme.

MATERIALS AND METHODS

Media. The synthetic medium A, the complex media AY, and LB and A-N buffer have been previously described (4). Solid media were prepared by the inclusion of 1.5% agar. Growth factors were added to medium A at a concentration of 100 μg/ml for amino acids and 10 μg/ml for thiamine, purines, and pyrimidines. The utilization of β-glucosides as carbon source was tested on solid medium A containing 0.5% arbutin, salicin, or β-methyl-glucoside.

Strains and nomenclature. The bacterial strains used are listed in Table 1. The bacteriophage used for transduction was P1. The origin and direction of entry of the Hfr strains are indicated in Fig. 1. The genotypes and phenotypes of the strains with regard to components of the β-glucoside system are given in Table 2.

Mutagenesis procedure. A 2-ml amount of an overnight culture was washed and suspended in 0.5 ml of 0.2 M sodium acetate buffer at pH 5 containing 50 μg of *N*-methyl-*N'*-nitro-nitrosoguanidine (NTG). After 2 h of incubation, 0.2 ml of culture was inoculated into 10 ml of LB broth and incubated for an additional 5 h. Then the cells were washed, diluted, and plated on selective media with salicin, arbutin, or β-methyl-glucoside as carbon source. Mutagenesis with ethyl methane sulfonate (EMS) was performed as follows: 0.05 ml of EMS was added to 2-ml samples of cells washed in A-N buffer. The cells were incubated for 30 min at 37 C. Further incubation in LB broth and selection were as described for NTG treatment.

Enzyme assays. The activity of *P*-β-glucosidase A in intact cells was determined by a method reported previously (4). The enzymatic assays with toluene-treated cells and with cell extracts were performed by using a coupled phosphorylating system consisting of G-6-*P* as phosphate donor, phosphatase-transphosphorylase (gl-phosphotransferase) from *Aerobacter aerogenes*, and PNP-glu as phosphate acceptor (7). The cells were grown in liquid AY medium supplemented with 0.5% sodium succinate to an optical density of 0.3 at 590 nm. For whole cells, 5 ml of suspension was centrifuged and the sedimented cells were washed twice with 0.075 M phosphate buffer at pH 6.8. Washed cells were suspended in 0.25 ml of buffer with 0.05 ml toluene and were incubated for 15 min at 35 C. Cell extracts were prepared by sonic oscillation. Cells were washed and suspended into phosphate buffer, ruptured three times for 45 s at intervals of 30 s with a Sonifier cell disruptor (Heat System Co., model 185C) at 120 W. The extracts were collected by centrifugation at 10,000 rpm for 20 min.

The reaction mixtures consisted of 0.25 ml of toluene-treated cells or sonic extracts, 0.2 ml of 4×10^{-2} M PNP-glu, 0.1 ml of 3×10^{-2} M G-6-*P*, and 0.1 ml of the gl-phosphotransferase preparation (7). The mixtures were incubated for 20 min at 35 C, and the reactions were stopped by the addition of 0.5 ml of Na_2CO_3. The liberation of *p*-nitrophenol was measured spectrophotometrically at 410 nm (Gilford 240 spectrophotometer). One unit of *P*-β-glucosidase activity was defined as the amount of enzyme which catalyzes the formation of 1 nmol of *p*-nitrophenol/min at 35 C. The protein content of the extracts was determined by the method of Lowry et al. (3).

Permease activity. Cells were washed twice and suspended in 0.075 M phosphate buffer at pH 6.8 to a final concentration of 0.4 mg (dry weight) of cells/ml. ^{14}C-thiophenyl-β-glucoside (specific activity, 4 mCi/mmol) was added to a final concentration of 2×10^{-4} M (20,000 counts/min). At different times, 1 ml of the suspension was removed, placed onto a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.), and washed with 4 ml of cold buffer. The filter was air-dried and radioactivity was determined in a thin-window counter (Nuclear Chicago Corp., Des Plains,

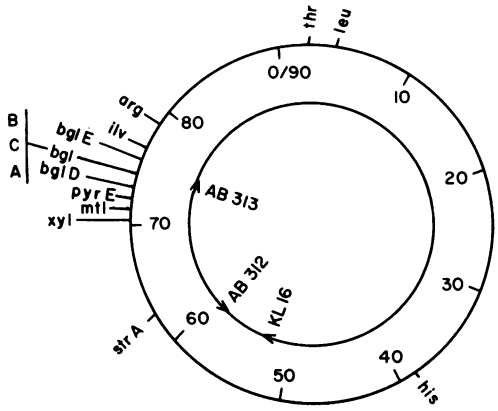

FIG. 1. *Genetic map of Escherichia coli showing the relative position of genetic loci used in this study. Map positions were obtained from references 5 and 8 and from the present study. Arrowheads on the inner circle indicate the points of origin and directions of chromosome transfer for the Hfr strains used.*

TABLE 1. *Bacterial strains used*

Strain	Genetic marker[a]	Origin
E. coli K-12 wild type	F^+ prototroph	(4)
AB 1450	F^- *arg, his, met, thi, ilv, xyl, str*	(4)
AB 312	Hfr thr^-, leu^-, thi^-, mtl^+, xyl^+, str^+	W. K. Maas
AB 313	Hfr thr^-, leu^-, thi^-, mtl^+, xyl^+, *str*	(4)
KL 16	Hfr prototroph str^+	W. K. Maas

[a] Genetic symbols are those described by Taylor (8).

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TABLE 2. *Genotype corresponding to the phenotypes of the β-glucosides system*[a]

Parental strain no.	Genotype					Mutation site in parental strain	Enzyme			Phenotype (fermentation)		
							P-β-glucosidase		Permease I	Arbutin	Salicin	β-Methyl-glucoside
	bglA	*bglB*	*bglC*	*bglD*	*bglE*		A	B				
1	+	−	+	+	+	WT	$+^{c}$	−	−	−	−	−
2	+	+	+	+	+	1 *bglB*	$+^{c}$	$+^{i}$	$+^{i}$	+	+	−
3	+	+	$+^{c}$	+	+	2 *bglC*	$+^{c}$	$+^{c}$	$+^{c}$	+	+	$-^{b}$
4	−	+	+	+	+	2 *bglA*	$+^{c}$	−	$+^{i}$	+	−	−
5	−	+	+	−	+	4 *bglD*	−	−	−	−	−	−
6	+	−	+	+	$+^{h}$	1 *bglE*	$+^{hc}$	−	−	−	−	+
7	−	+	$+^{c}$	+	+	3 *bglA*	+	−	$+^{c}$	+	−	−
8	−	+	$+^{c}$	−	+	5 *bglC*	−	−	$+^{c}$	−	−	−

[a] Abbreviations: *bglA*, structural gene for *P-β*-glucosidase B; *bglB*, regulatory site for β-glucoside permease I and *P-β*-glucosidase B; *bglD*, structural gene for *P-β*-glucosidase A; *bglC*, regulatory gene or site for *P-β*-glucosidase B and β-glucoside permease I; *bglE*, regulatory gene or site for *P-β*-glucosidase A; [c], constitutive allele for *P-β*-glucosidase B and β-glucoside permease I; [h], increase of constitutive *P-β*-glucosidase A activity; [c], constitutive biosynthesis; [i], inducible biosynthesis; [b], slight background growth on β-methyl-glucoside; WT, wild type.

Ill.). Uptake was expressed in micromoles per gram (dry weight) of bacteria. "Chasing" experiments were performed by the addition of β- and α-glucosides at a final concentration of 5×10^{-3} M.

Conjugation and transduction. The procedures were the same as reported previously (5).

RESULTS

P-β-glucosidase A-negative mutants. The wild-type cells of *E. coli* possess constitutive *P-β*-glucosidase A activity but lack *P-β*-glucosidase B and β-glucoside permease I activities. Because of the absence of permease I, the intact cells fail to express the activity of *P-β*-glucosidase A and thus do not ferment arbutin (6, 7). This lack of phenotypic expression precludes the direct isolation of mutants lacking *P-β*-glucosidase A. However, such mutants were obtained from strain AB 1450 in three successive steps (Table 2). In step 1, the activities of *P-β*-glucosidase B and β-glucoside permease I were expressed as a result of mutation at the regulatory site *bglB* (5; Prasad and Schaefler, unpublished data): AB 1450/1, wild type, $bglD^{+}$, $bglA^{+}$, $bglC^{+}$, $bglB^{-} \xrightarrow{\text{spontaneous}}$ AB 1450/2 $bglD^{+}$, $bglA^{+}$, $bglC^{+}$, $bglB^{+}$. The mutant grows on medium containing arbutin or salicin as carbon source. In step 2, the activity of *P-β*-glucosidase B was eliminated by a mutation at *bglA*, the structural gene for *P-β*-glucosidase B. However, the activities of *P-β*-glucosidase A ($bglD^{+}$) and β-glucoside permease I ($bglB^{+}$) were preserved: AB 1450/2 $bglD^{+}$, $bglA^{+}$, $bglC^{+}$, $bglB^{+} \xrightarrow{\text{EMS}}$ AB 1450/4 $bglD^{+}$, $bglA^{-}$, $bglC^{+}$, $bglB^{+}$. The mutant grows on arbutin but not on salicin medium. In step 3, the activity of *P-β*-glucosidase A was eliminated by a mutation at the site of *bglD* which apparently determines the constitutive biosynthesis of *P-β*-glucosidase A: AB 1450/4 $bglD^{+}$, $bglA^{-}$, $bglC^{+}$, $bglB^{+} \xrightarrow{\text{EMS}}$ AB 1450/5 $bglD^{-}$, $bglA^{-}$, $bglC^{+}$, $bglB^{+}$. The mutant does not grow on arbutin or salicin medium.

At step 3, the mutation occurred apparently at a very low frequency. Among 208 arbutin-negative mutants isolated from strain AB 1450/4, only two (AB 1450/5 and AB 1450/5A) showed no detectable *P-β*-glucosidase A activity after cell lysis by toluene treatment and addition of phosphorylated PNP-glu. The remainder were probably permease-negative mutants. Repeated attempts with other *E. coli* K-12 derivatives gave negative results. The extracts of the mutants AB 1450/5 and 5A showed no detectable *P-β*-glucosidase A or B activities. These mutants also had a reduced rate of inducible permease activity. Uptake experiments with ^{14}C-thiophenyl-β-glucoside (TPG), which is accumulated by β-glucoside permease I (1, 4, 7), indicated that after growth in the presence of 5×10^{-3} M salicin strain AB 1450/5 showed a much lower level of accumulation of TPG than the parental strain AB 1450/4 (Fig. 2). It is assumed that the low level of accumulation is due to the lack of induction of permease I activity, although an increased exit activity cannot be excluded. ^{14}C-TPG accumulated by induced cells of the strains AB 1450/4 and 1450/5 was chased by phenyl-β-glucoside and β-methyl-glucoside, but not by α-methyl-glucoside, glucose, or cellobiose. It appears, therefore, that the low permease activity induced in

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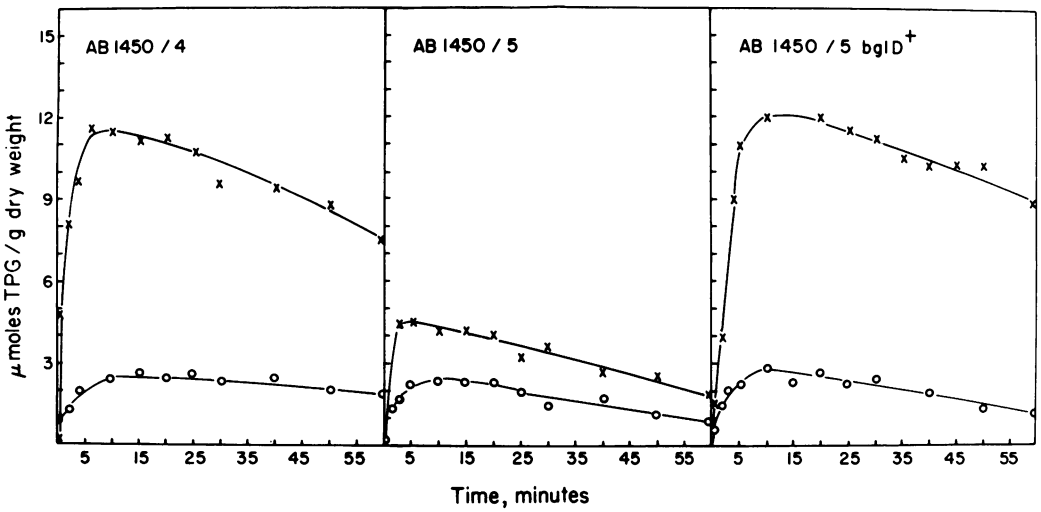

FIG. 2. *Uptake of ^{14}C-thiophenyl β-glucoside by noninduced cells and cells induced by salicin. Cultures of AB 1450/4, AB 1450/5, and AB 1450/5 bglD$^+$ were grown in AY medium (noninduced) and medium with 5 × 10^{-3} M salicin (induced). Washed cells (400 g of dry weight/ml) were suspended in buffer with ^{14}C-TPG (specific activity, 4 mCi/mmol) at a concentration of 2 × 10^{-4} M (20,000 counts/min). The uptake was determined at 28 C. At different times the accumulated TPG was determined. (×) Induced cells; (O) noninduced cells.*

strain AB 1450/5 has a substrate specificity similar to that previously described for β-glucoside permease I (4, 7).

P-β-glucosidase A-positive back-mutants of strain AB 1450/5 regained fully inducible β-glucoside permease I activity (Fig. 2). The permease activity was also restored after transfer by transduction of the structural gene for *P-β*-glucosidase A or B. After introduction of *P-β*-glucosidase B, the maximal activity upon induction by TPG was 11.6 μmol/g (dry weight). Introduction in strain AB 1450/5 of the constitutive allele *bglC^c* of the regulatory gene *bglC*, without simultaneous transfer of *P-β*-glucosidase A or B, resulted in high constitutive uptake of TPG (12 μmol/g, dry weight). It appears, therefore, that the presence of one of the *P-β*-glucosidases is required for the induction but not for the function of the β-glucoside permease I activity.

Mutants with increased P-β-glucosidase A activity. β-Methyl-glucoside can be accumulated in cells by both the glucose permease and β-glucoside permease I. Yet, because of the relatively low affinity of *P-β*-glucosidase A for phosphorylated β-methyl-glucoside, wild-type cells which possess constitutive *P-β*-glucosidase A and glucose permease show no detectable growth on synthetic medium containing 0.5% β-methyl-glucoside as carbon source. β-Methyl-glucoside-fermenting mutants were induced by EMS in wild-type cells and were isolated on synthetic medium with β-methyl-glucoside as carbon source. Such mutants showed an increased level of *P-β*-glucosidase A activity, but no detectable *P-β*-glucosidase B and β-glucoside permease I activities (6). Extracts of *P-β*-glucosidase A hyperproducers (phenotype β-met$^+$) with phosphorylated PNP-glu as substrate showed an activity of 49 to 82 units/mg of protein as compared to 8 units/mg of wild-type extracts. The enzyme was not inactivated by incubation for 30 min at 50 C and had a pH optimum of 6.8. The enzyme did not hydrolyze phosphorylated salicin and showed kinetic and chromatographic characteristics similar to those of *P-β*-glucosidase A of wild-type cells (G. Wilson and F. Fox, in preparation). Because the intact cells of the β-met$^+$ mutant lacked β-glucoside permease I, they did not hydrolyze PNP-glu, arbutin, and other aromatic β-glucosides. Genetic data have shown that a mutation at a regulatory gene (*bglE$^+$ → bglE^h*) determines an increased level of *P-β*-glucosidase A activity.

The β-met$^+$ mutants studied so far were relatively unstable. After several subcultures in LB broth, the majority of the bacterial population consisted of cells with a wild-type level of enzymatic activity. In addition, back-mutants with an intermediate level of enzymatic activity were also obtained. These data appear to indicate that the cells with a wild-type level of enzyme possess selective advantages over hyperproducers.

Mapping of the bglD gene. By successive

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mutations (5), the wild-type prototrophic strain *E. coli* K-12 acquired the genotype *bglD*$^+$, *bglA*$^-$, *bglC*^{c}, *bglB*$^+$. The strain hydrolyzes arbutin but not salicin because of the presence of *P*-β-glucosidase A and the absence of *P*-β-glucosidase B. The strain is constitutive for β-glucoside permease I. The genetic markers *bglA*$^-$, *bglC*^{c}, and *bglB*$^+$ were transduced into Hfr strains with wild-type *bgl* genotypes AB 313, AB 312, and KL 16 (Fig. 1), where the *bglD*$^+$ allele is present, yielding the derivatives AB 313/7, AB 312/7, and KL 16/7 (Table 2). These Hfr strains were mated with strain AB 1450/5 (*bglD*$^-$, *bglA*$^-$, *bglC*$^+$, *bglB*$^+$). Table 3 shows that the Hfr strains AB 312/7 and AB 313/7 transferred the *bglD*$^+$ gene at high frequency to the F$^-$ strain AB 1450/5 when *ilv*$^+$ and *xyl*$^+$ colonies were selected, respectively. Upon selection of *his*$^+$ colonies in the cross between AB 313/7 and AB 1450/5, the number of arbutin-fermenting colonies was similar to those scored when *xyl*$^+$ colonies were screened. However, when *his*$^+$ colonies were selected in the cross of KL 16/7 × AB 1450/5, no arbutin-fermenting (*bglD*$^+$) colonies or *ilv*$^+$ colonies were found. Among the arbutin-fermenting recombinants (*bglD*$^+$) from the cross of AB 1450/5 with AB 312/7 and with AB 313/7, 96 and 93% were permease-constitutive (*bglC*^{c}), respectively, thus indicating that *bglD* is located close to the *bglC*^{c} gene which was previously mapped at 73 min on the *E. coli* map (8).

Additional data on the location of the *bglD* locus were obtained by transduction with the P1 phage. Table 4 (part A) shows that the *bglD* locus co-transduces with the *bglC* locus at a higher frequency and with the *bglE* locus, which is closer to the *ilv* than to the *bglC* locus, at a lower frequency. No co-transduction with the *ilvD* gene was obtained after the selection of 1,600 *ilv*$^+$ recombinants. The co-transduction frequency of *bglD*$^+$ with *pyrE* was very low, whether the selection was made for *bglD*$^+$ or *pyrE*$^+$. Additional experiments indicated that *bglD* does not co-transduce with *mtl*. The above results are consistent with the data obtained by conjugation and indicated that *bglD* is located between *pyrE* and the cluster of *bgl* genes.

Mapping of the bglE^h locus. The mapping of the locus determining hyperproduction of *P*-β-glucosidase A was made by isolating *bglE*^{h} mutants from the Hfr strains AB 312 and AB 313 and mating these strains with strain AB 1450. The results of conjugation experiments 2 and 4 in Table 3 indicate that strain AB 313/6 *bglE*^{h} did not transfer the *bglE*^{h} gene into AB 1450/4 when selection was made for *xyl*$^+$. However, when the strain AB 312/6 *bglE*^{h} was used as donor and selection was made for *ilv*$^+$ (Table 3), 64% of the colonies were β-methyl-glucoside-fermenting colonies (*bglE*^{h}). The above data indicate that the *bglE* locus is located between the origin of Hfr AB 313 and the cluster of *ilv* genes. These data were confirmed by the results of transduction experiments (Table 4, part B, experiment 2), which show that, upon selection of *ilvD*$^+$, *bglE* was transduced at a frequency of 90%, whereas the previous data indicate that *bglB* was co-transduced with *ilvD*$^+$ at a frequency of 28% (5). The co-transduction frequency of *bglE* with *bglB* was 22% and that with

TABLE 3. *Mapping by conjugation of the bglD and bglE genes*

Expt. no.	Donor Hfr	Recipient F$^-$	Selected marker	No. of colonies[a]		
				Arb$^+$ (*bglD*$^+$)	Const (*bglC*^{c}, *B*$^+$)	β-Met$^+$ (*bglE*^{h})
1	AB 313/7[b] *bglD*$^+$, *A*$^-$, *C*^{c}, *B*$^+$, *E*$^+$	AB 1450/5 *bglD*$^-$, *A*$^-$, *C*$^+$, *B*$^+$, *E*$^+$	*his*$^+$	114	82	—
			xyl$^+$	160	150	—
2	AB 313/6 *bglD*$^+$, *A*$^+$, *C*$^+$, *B*$^-$, *E*^{h}	AB 1450/4 *bglD*$^+$, *A*$^-$, *C*$^+$, *B*$^+$, *E*$^+$	*xyl*$^+$	140	—	0
3	AB 312/7 *bglD*$^+$, *A*$^-$, *C*^{c}, *B*$^+$, *E*$^+$	AB 1450/5 *bglD*$^-$, *A*$^-$, *C*$^+$, *B*$^+$, *E*$^+$	*ilv*$^+$	178	172	—
4	AB 312/6 *bglD*$^+$, *A*$^+$, *C*$^+$, *B*$^-$, *E*^{h}	AB 1450/4 *bglD*$^+$, *A*$^-$, *C*$^+$, *B*$^+$, *E*$^+$	*ilv*$^+$	20	—	128
5	KL 16/7 *bglD*$^+$, *A*$^-$, *C*^{c}, *B*$^+$, *E*$^+$	AB 1450/5 *bglD*$^-$, *A*$^-$, *C*$^+$, *B*$^+$, *E*$^+$	*his*$^+$	0	0	—

[a] The total number scored for each marker was 200.

[b] Number refers to the parental strain number in Table 2. Arb$^+$, colony fermenting arbutin; Const, constitutive permease; β-Met$^+$, colony fermenting β-methyl-glucoside; —, not tested.

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TABLE 4. *Mapping by transduction of the structural and regulatory genes for P-β-glucosidase A*[a]

Part[b]	Expt no.	Donor	Recipient	Genotype selected	Frequency of unselected marker inherited/no. of colonies analyzed
A	1	Prototroph/4[c] *bglD*$^+$,*A*$^-$,*C*$^+$,*B*$^+$,*E*$^+$	AB 1450/8 *bglD*$^-$,*A*$^-$,*C*^{c},*B*$^+$,*E*$^+$	*bglD*$^+$	80, *bglC*$^+$/200
	2	Prototroph/4 *bglD*$^+$,*A*$^-$,*C*$^+$,*B*$^+$,*E*$^+$	AB 1450/5 *bglD*$^-$,*A*$^-$,*C*$^+$,*B*$^+$,*E*$^+$	*ilv*$^+$	0, *bglD*$^+$/1,600
	3	Prototroph/6 *bglD*$^+$,*A*$^+$,*C*$^+$,*B*$^-$,*E*^{n}	AB 1450/5 *bglD*$^-$,*A*$^-$,*C*$^+$,*B*$^+$,*E*$^+$	*bglD*$^+$	20, *bglE*^{n}/200
	4	AB 1450/8 *bglD*$^-$,*A*$^-$,*C*^{c},*B*$^+$,*E*$^+$	AB 1450 *pyrE*$^-$/7 *bglD*$^+$,*A*$^-$,*C*^{c},*B*$^+$,*E*$^+$	*pyrE*$^+$	2, *bglD*$^-$/200
	5	AB 1450/7 *bglD*$^+$,*A*,*C*^{c},*B*$^+$,*E*$^+$	AB 1450 *pyreE*$^-$/8 *bglD*$^-$,*A*$^-$,*C*^{c},*B*$^+$,*E*$^+$	*bglD*$^+$	5, *pyrE*$^+$/200
B	1	Prototroph/4 *bglD*$^+$,*A*$^-$,*C*$^+$,*B*$^+$,*E*$^+$	Prototroph/6 *bglD*$^+$,*A*$^+$,*C*$^+$,*B*$^-$,*E*^{n}	*bglB*$^+$	44, *bglE*$^+$/200
	2	Prototroph/6 *bglD*$^+$,*A*$^+$,*C*$^+$,*B*$^-$,*E*^{n}	AB 1450/4 *bglD*$^+$,*A*$^-$,*C*$^+$,*B*$^+$,*E*$^+$	*ilv*$^+$	180, *bglE*^{n}/200

[a] All of the transductants growing on selective plates were subcultured to test the stability of the colonies and the unselected markers. The Arb$^+$, Arb$^-$, β-met$^+$, and β-met$^-$ colonies were individually tested for the presence of enzyme. With β-met$^+$ colonies, the level of enzyme was always higher than the control.

[b] A, mapping of *bglD*, B, mapping of *bglE*.

[c] Number refers to the parental strain number in Table 2.

bglD was 10% (Table 4, experiment 2 in part B and 3 in part A). This indicates that *bglE* is located between the *ilv* and *bgl* loci, being closer to the *ilvD* than to the *bglB* locus.

DISCUSSION

P-β-glucosidase A is synthesized constitutively by *E. coli* K-12 and other strains of *E. coli* (6). The enzyme has a high affinity for phosphorylated aromatic β-glucosides, with the exception of phosphorylated salicin, and a low affinity for phosphorylated β-methyl-glucoside. The activity of the enzyme is not expressed in intact wild-type cells because of the lack of β-glucoside permease I. Although β-methyl-glucoside is accumulated by the glucose permease of wild-type cells, the low affinity of the enzyme for phosphorylated β-methyl-glucoside precludes the growth on media with β-methyl-glucoside as carbon source. In wild-type strains of *E. coli*, the activity of the enzyme can be detected only by using toluene-treated cells or extracts and phosphorylated PNP-glu as substrate. *P*-β-glucosidase A apparently has only a very limited role in the utilization of external β-glucosides, and the actual physiological function of this cryptic constitutive enzyme is still unknown.

In the present study, we investigated two types of mutants in which the activity of *P*-β-glucosidase A was altered: (i) mutants lacking the ability to synthesize *P*-β-glucosidase A, apparently because of a genetic block in the structural gene for *P*-β-glucosidase A (*bglD*), and (ii) *P*-β-glucosidase A hyperproducers resulting from a mutation in a regulatory gene (*bglE*), which increases the rate of biosynthesis of the enzyme.

The mutation leading to the loss of *P*-β-glucosidase A activity occurred at a very low frequency and was obtained only from a derivative of the strain AB 1450. We were unable to obtain similar mutants from other *E. coli* K-12 derivatives. It could be that the inability to obtain *bglD*$^-$ mutants in strains other than AB 1450 is due to the presence of an additional compensating mutation in the strain AB 1450. This possibility requires further investigation.

One of the physiological functions of *P*-β-glucosidase A appears to be related to the induction of β-glucoside permease I. Previous data obtained with strains possessing *P*-β-glucosidase A and inducible β-glucoside permease I indicated that nonmetabolizable thioglucosides, such as thiomethyl β-glucoside, can serve as inducers (4); it appears therefore improbable that the effect of the presence of *P*-β-glucosidase A on the inducibility of β-glucoside permease I is a result of the hydrolytic activity of the enzyme.

It was previously found that the *bgl* loci lie between the *pyrE* and the *ilv* loci, their sequence being *pyrE*, *bglA*, *bglC*, *bglB*, origin of Hfr AB 313, and the *ilv* loci. The present data indicate the location of *bglD* between *pyrE* and *bglA* and of *bglE* between the origin of Hfr AB 313 and the *ilv* loci; the tentative order is therefore *pyrE*, *bglD*, *bglA*, *bglC*, *bglB*, origin of AB 313, *bglE*, and *ilv*. No data are yet available

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on the relationship of the *bgl* loci to other loci situated between *pyrE* and the *ilv* cluster (8). The position of *bglD* between *pyrE* and the *bgl* cluster was determined by its co-transduction with *pyrE* and the lack of co-transduction with *ilvD*. In contrast to *bglD*, all other *bgl* loci are co-transduced with *ilvD* at different frequencies (5). The higher rate of co-transduction of *bglD* with *bglC* than with *bglE* is consistent with the positions assigned to these loci. The analysis of the co-transduction of *bglD* with *bglA* is technically difficult and is under investigation. The regulatory gene *bglE* is located between the origin of AB 313 and the *ilv* loci. This location was demonstrated by the transfer of *bglE* by conjugation with the strain Hfr AB 312 but not with the strain Hfr AB 313 and by the higher rate of co-transduction with *ilvD* of the *bglE* locus than of either the *bglB* or *bglA* loci.

The hyperproduction of *P*-β-glucosidase A is determined by a mutation in the regulatory gene *bglE*. The data of Wilson and Fox (personal communication) seem to indicate that the increase of *P*-β-glucosidase A activity is due to an increase in the quantity of enzyme present in the bacterial cell and not to a change in the characteristics of the enzyme. The relative instability of the hyperproducers appears to indicate that wild-type cells contain an optimal level of the enzyme which confers the selective advantages over the hyperproducers. The genetic mechanism that governs the constitutive hyperproduction of *P*-β-glucosidase A is still a matter of speculation. The presence of the *bglA*, *bglC*, and *bglB* sites between *bglD* and *bglE* and the lack of effect of the mutation toward hyperproduction on the activity of *P*-β-glucosidase B and β-glucoside permease I makes it improbable that the $bglE^+ \rightarrow bglE^h$ mutation occurs at a promoter site, as was assumed for hyperproduction of glucose-6-phosphate dehydrogenase (2). It appears more probable that *bglE* determines the biosynthesis of a soluble product that affects the rate of biosynthesis of *P*-β-glucosidase A.

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