JOURNAL OF BACTERIOLOGY, Nov. 1972, p. 840-848
Vol. 112, No. 2

Reversal of the Mannitol-Sorbitol Diauxie in *Escherichia coli*

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Received for publication 11 May 1972

In *Escherichia coli* K-12 the proteins involved in the dissimilation of mannitol and sorbitol are specified by two separate gene clusters. The mannitol cluster appears to consist of a regulatory gene *mtlC*, a gene *mtlA* coding an enzyme II complex of the phosphoenolpyruvate phosphotransferase system, and a gene *mtlD* coding a mannitol-1-phosphate dehydrogenase. Three corresponding genes, *sblC*, *sblA*, and *sblD*, exist for the sorbitol pathway. In both pathways the hexitol captured from the medium and delivered into the cytoplasm as a phosphorylated compound is dehydrogenated to fructose-6-phosphate. The enzyme II complex for sorbitol is able to catalyze the phosphorylation also of mannitol if this substrate is present at high concentrations. Consequently *mtlA*$^-$ mutants lacking the enzyme II complex for mannitol can grow on mannitol either if the sorbitol phosphorylating system is preinduced by sorbitol or if *mtlA* is suppressed by a mutation of *sblC* to constitutivity. In wild-type cells, the induction of the enzymes in the mannitol pathway and dissimilation of the substrate are not prevented by glucose. The sorbitol system, however, is sensitive to glucose and to mannitol as well. In the suppressed strains (*mtlA*$^-$, *sblC*^{c}) in which mannitol is utilized through the sorbitol enzyme, glucose becomes effective in restraining the consumption of mannitol, causing a definite diauxie. Moreover, in a mixture of mannitol and sorbitol, the latter is utilized preferentially. This reversal of normal diauxic pattern is consequent to the fact that the enzyme II complex for sorbitol has relatively poor affinity for mannitol.

The catabolism of mannitol is initiated in *Escherichia coli* K-12 by a phosphoenolpyruvate (PEP) phosphotransferase-mediated phosphorylation (18-20).

$$\text{HPr} + \text{PEP} \underset{\text{Mg}^{2+}}{\overset{\text{enzyme I}}{\rightleftharpoons}} \text{P-HPr} + \text{pyruvate} \quad (1)$$

$$\text{P-HPr} + \text{mannitol} \xrightarrow{\text{enzyme II complex}_{mtl}} \text{mannitol-1-P} + \text{HPr} \quad (2)$$

HPr is a low-molecular-weight histidine-containing protein, and P-HPr is its high-energy phosphate derivative. The enzyme II complex is membrane-associated and is responsible both for the capture of the substrate from the medium and for its phosphorylation. Mannitol-1-phosphate appearing in the cytoplasm is then dehydrogenated by a nicotinamide adenine dinucleotide (NAD)-dependent enzyme to yield fructose-6-phosphate:

$$\text{Mannitol-1-P} + \text{NAD} \overset{\text{mannitol-1-P dehydrogenase}}{\rightleftharpoons} \text{fructose-6-P} + \text{NADH} \quad (3)$$

During our study of a mutant blocked in reaction 2 and therefore unable to grow on manntiol, a revertant was isolated which was able to grow on this carbon source at high concentrations but was not restored in the missing enzyme II complex$_{mtl}$. The suppression has been diagnosed as a regulatory mutation leading to the constitutive expression of the sorbitol pathway. Previous studies have revealed that sorbitol is also dissimilated via the PEP-phosphotransferase system, but by a separate enzyme II complex. This was demonstrated directly in the case of *Klebsiella* (or *Aerobacter*) *aerogenes* (8) and was inferred in the case of *E. coli* (20, 23), a conclusion which we confirm. The mechanism by which constitutivity of the sorbitol pathway allows a cell to

bypass the block in the first step of normal mannitol metabolism, as well as the consequence on the order of utilization of mannitol in the presence of glucose and sorbitol, will be described.

MATERIALS AND METHODS

Chemicals. Mannitol-1-phosphate, sorbitol-6-phosphate, sodium phosphoenolpyruvate, [3,-(4,5-dimethyl thiazolyl-2)-2,5-diphenyltetrazolium bromide] (MTT), phenazine methosulfate, lysozyme from egg white (grade I), deoxyribonuclease from beef pancreas, and sorbitol dehydrogenase from sheep liver were obtained from Sigma Chemical Co., St. Louis, Mo. ^{14}C-mannitol, ^{3}H-mannitol, and ^{14}C-sorbitol were obtained from New England Nuclear Corp., Boston, Mass. Casein acid hydrolysate (salt free, vitamin free) was from Nutritional Biochemicals, Cleveland, Ohio.

Bacterial strains. The origins and genotypes of the mutants used in this study are summarized in Table 1. Strain 157 (F^-, *strA*, *xyl*$^-$, *lct*$^-$, *mtlA*$^-$, *ilv*$^-$, *thi*$^-$, *argH*$^-$) was from J. Puig (17, 19). Strain 158, a spontaneous mannitol-positive revertant of 157, was isolated by E. Solomon. Strain 159 was constructed by transduction of the *xyl*$^+$ (selected) and *mtl*$^+$ (unselected) markers from the wild-type strain 1 (25) into strain 157.

Culture media and growth conditions. To a mineral medium (21), the following additions were made as necessary: casein hydrolysate at a final concentration of 1%; single carbon sources at 0.2%, except for the diauxie experiments where each was added at 0.03%. Growth at 37 C of a 10-ml culture in a 250-ml side-arm flask was determined turbidimetrically with a Klett colorimeter (filter 42). For an exponential culture, 20 Klett units equal 10^8 bacteria/ml.

Phage P1 transduction. The procedure was as described by Arber (2).

Uptake assay. Exponentially growing cells were harvested from a culture at 100 Klett units by centrifugation. They were then washed twice at room temperature with mineral medium and resuspended to a density of about 20 Klett units. To 0.9 ml of this suspension, kept at 25 C in a Wasserman tube, was added 0.1 ml of ^{3}H-mannitol (12.5 Ci/mole, 5×10^{-6} M) or 0.1 ml of ^{14}C-sorbitol (12.5 Ci/mole, 5×10^{-6} M). After 30 sec, 5 ml of ice-cold mineral medium was added, and the cells were collected on a membrane filter (0.65-μm pore diameter) by filtration and washed with 5 ml of cold medium. The amount of radioactivity on the dried filter was determined in a scintillation counter. Uptake activities are expressed in nanomoles per minute per milligram of protein.

Preparation of cell-free extracts. Cells were grown to 250 Klett units in a 100-ml culture, harvested, washed twice with ice-cold 1% NaCl solution, resuspended in 1 ml tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (0.1 M, *p*H 7.6), and sonically treated for 1 min in a model 60W MSE apparatus (Measuring and Scientific Equipment Ltd., London, England) while being chilled in a −20 C bath. The disrupted preparation was centrifuged at 30,000 × *g* for 20 min, and the supernatant fraction was collected for enzyme assays.

The enzyme II complex activities as well as the dehydrogenase activities, are stable to repeated freezing and thawing, but enzyme I activity is not. Strain 236 (19), lacking the enzyme II complex$_{mtl}$ activity because of a mutation, and lacking the enzyme II complex$_{sbl}$ activity because of repression resulting from growth on glucose, was used as the source for enzyme I and HPr (21). A different procedure of cell disruption was found useful in eliminating a slight nonspecific sorbitol phosphorylating activity not related to enzyme II complex$_{sbl}$ in the following manner. Harvested cells were incubated in 1 mM mercaptoethanol and 0.25 mM ethylenediaminetetraacetate for 10 min at 28 C with gentle shaking. A 2-mg amount of crystalline lysozyme was then added, and 25 min later 0.08 ml of deoxyribonuclease solution (1 mg/ml in 0.5 M $MgCl_2$) was introduced. After 5 min, the extract was chilled and centrifuged for 20 min at 30,000 × *g*

Assay of enzyme II complex$_{mtl}$. The activity was measured by following the rate of mannitol-1-phosphate formation from ^{14}C-mannitol (21). The assay mixture contained in a total volume of 0.2 ml: ^{14}C-mannitol (2.5 Ci/mole, 5×10^{-5} M); PEP (5×10^{-3} M; $MgCl_2$ (5×10^{-5} M); enzyme I and HPr in an extract of strain 236 cells (18 mg/ml of protein); and Tris-hydrochloride buffer (0.1 M, *p*H 7.6). After a 5-min equilibration at 25 C, the reaction was started

TABLE 1. *Origin and genotypes of strains of E. coli K-12*

Strain	Parent	Isolation	Mannitol markers[a]			Sorbitol markers		
			mtlC	*mtlA*	*mtlD*	*sblC*	*sblA*	*sblD*
157	560		+	−	+	+	+	+
158	157	Mtl$^+$ revertant	+	−	+	c	+	+
159	157	*mtl*$^+$ transductant	+	+	+	+	+	+
238	AB313		c	+	+	+	+	+

[a] *mtlC* designates the gene determining inducibility or constitutivity of the mannitol pathway; *mtlA*, the gene specifying the enzyme II complex for mannitol; *mtlD*, the gene specifying mannitol-1-phosphate dehydrogenase. A similar set of symbols is employed for the genes of the sorbitol pathway. The symbol Mtl$^+$ is used to describe a mannitol-positive phenotype, the symbol *mtl*$^+$ to describe the mannitol-positive genotype. Genetic symbols are according to Taylor (22).

by the addition of the sample to be assayed. At 5, 10, and 15 min, 0.05-ml samples were delivered on a diethylaminoethyl paper disc which was immediately dropped in 80% ethanol. The filters were washed three times with deionized water and dried, and the radioactivity was determined in a scintillation counter. Enzyme II $complex_{mtl}$ activities are expressed in nanomoles per minute per milligram of protein.

Assay of enzyme II $complex_{sbl}$. The assay was similar to that described for enzyme II $complex_{mtl}$ except ^{14}C-sorbitol (2.5 Ci/mole, 5×10^{-5} M) was used as the substrate.

Assay of mannitol-1-phosphate dehydrogenase. The activity was measured at 30 C by following the rate of NAD reduction at 340 nm (23). The reaction mixture contained in a total volume of 0.5 ml: 0.1 ml of mannitol-1-phosphate (10^{-2} M); 0.05 ml of NAD (2×10^{-2} M); 0.35 ml of Tris-hydrochloride buffer (1 M, *p*H 9.0); and cell extract. Tris-hydrochloride rather than carbonate buffer was used because the latter appears to inhibit the enzyme slightly (8). The activity is expressed in micromoles per minute per milligram of protein.

Assay of sorbitol-6-phosphate dehydrogenase. The activity was measured by reduction of a tetrazolium dye (21). The assay mixture contained in a total of 0.5 ml: 0.03 ml of mixture A (containing 0.33 ml of Tris-hydrochloride, 0.1 M at *p*H 7.5; 0.07 ml of MTT [0.5 mg/ml]; 0.01 ml of Triton X-100 [20%, v/v]; 0.3 ml of water); 0.05 ml of NAD (2×10^{-2} M); 0.05 ml of phenazine methosulfate (1 mg/ml); 0.1 ml of sorbitol-6-phosphate (10^{-2} M). The reaction was started by adding sonic extract, and the optical density change at 570 nm was followed. All commercially available sorbitol-6-phosphate contained up to 5% mannitol-1-phosphate. In extracts containing significant levels of both mannitol-1-P and sorbitol-6-P dehydrogenases, the latter could be properly measured after the contaminating mannitol-1-P was removed by preincubation with a preparation containing only mannitol-1-P dehydrogenase. The activity is expressed in micromoles per minute per milligram of protein.

Quantitation of mannitol. The D-arabitol dehydrogenase from *Klebsiella aerogenes* has been shown to act also on mannitol but not on sorbitol (12), and thus it can be used to determine the mannitol concentration in the growth medium. The enzyme was prepared as previously described (12) from a strain producing the arabitol dehydrogenase constitutively. The enzyme precipitated after the first ammonium sulfate step was redissolved in Tris-hydrochloride buffer (0.1 M, *p*H 7.6). It had a specific activity of 3.3 units/mg of protein. The assays of mannitol concentration were carried out essentially under the same conditions described for the sorbitol-6-phosphate dehydrogenase assay except 0.31 unit of partially purified D-arabitol dehydrogenase was used. The unknown sample was diluted to 10^{-6} to 4×10^{-5} M mannitol in the assay mixture. The plateau of the optical density at 570 nm was reached between 10 to 25 min. A reference solution of mannitol, twice recrystallized from water and containing less than 0.16% sorbitol as impurity, was standardized by the method of Korn (9) based on formaldehyde units.

Quantitation of sorbitol. The sorbitol concentration in growth media was determined by a procedure similar to the one described above, except 0.5 unit of sorbitol dehydrogenase (from sheep liver, 25 units/mg of protein) in the assay mixture was used. The enzyme does not act on mannitol. Commercial sorbitol (which can contain up to 2% mannitol) may therefore serve as a standard without further purification.

RESULTS

Nature of the mannitol revertant strain 158. Strain 158 was isolated from strain 157, a mutant lacking enzyme II $complex_{mtl}$, as a revertant on a minimal mannitol plate containing 0.2% of the carbon source. Unlike other revertants studied, this strain was restored neither in its enzyme II $complex_{mtl}$ nor in its ability to capture the substrate from the medium at low concentrations (Table 2). Subsequent exploration revealed that the enzymes in the sorbitol pathway had become constitutive. In wild-type cells, the pathway for each hexitol is induced by its respective substrate (Tables 2 and 3). It would seem that enzyme II $complex_{sbl}$ has some affinity for mannitol but cannot intervene in the mutant strain 157 to take over the lost function because mannitol is unable to induce the enzyme. This hypothesis is supported by the observation that extracts of strain 157 and 159 were more effective in catalyzing the phosphorylation of mannitol when grown on glycerol plus sorbitol than on glycerol alone. It might be noted also that mannitol-1-phosphate dehydrogenase activity was not affected by growth in the presence of sorbitol, indicating that the increase in mannitol phosphorylating activity is not attributable to a change in the bona fide mannitol pathway. The contribution of the sorbitol system on mannitol metabolism was not as clearly manifested in uptake assays by intact cells, presumably because at the very low concentration of mannitol used (one-tenth of that employed for the phosphorylation assay) little engagement of the sorbitol enzyme could take place because of poor affinity. Finally, the phosphorylating activity of extracts of strain 158 on ^{14}C-mannitol was inhibited more than 10-fold in the presence of 5×10^{-5} M unlabeled sorbitol (data not shown).

Genetic confirmation of our interpretation was provided by P1 transduction of the suppressor locus into other *mtlA*$^-$ mutants, which showed that the suppression is not linked to the *mtl* marker, but to *sbl* (data on the map will be reported elsewhere). Moreover, all

TABLE 2. *Uptake and enzyme activities for mannitol*[a]

Strain[b]	Inducer added	Mannitol uptake[c] (nmoles/min/mg of protein)	Enzyme II complex$_{mtl}$[d] (nmoles/min/mg of protein)	Mannitol-1-P dehydrogenase (μmoles/min/mg)
157 (Mannitol negative)		1.4	0.1	0.2
	Mannitol	0.7	0.3	0.4
	Sorbitol	1.2	1.2[e]	0.3
158 (Mannitol "revertant")		1.3	1.0[e]	0.2
	Mannitol	0.6	1.6[e]	0.3
	Sorbitol	1.0	1.6[e]	0.1
159 (Wild type)		19	0.8	0.1
	Mannitol	67	8.3	1.3
	Sorbitol	25	2.3[e]	0.3
238 (Mannitol constitutive)		116	21	2.8
	Mannitol	81	13	2.4

[a] Cells were grown in minimal medium + 0.2% glycerol with or without 0.2% hexitol as indicated. Detailed conditions for the assays of uptake and enzymes are given in Materials and Methods.
[b] Markers not specified are wild-type alleles.
[c] 5×10^{-6} M mannitol.
[d] 5×10^{-5} M mannitol.
[e] Activity partially attributable to enzyme II complex$_{sbl}$.

TABLE 3. *Uptake and enzyme activities for sorbitol*[a]

Strain[b]	Inducer added	Sorbitol uptake[c] (nmoles/min/mg of protein)	Enzyme II complex$_{sbl}$[d] (nmoles/min/mg of protein)	Sorbitol-6-P dehydrogenase (μmoles/min/mg)
157 (Mannitol negative)		1.7	0.07	0.01
	Mannitol	4.4	0.09	0.03
	Sorbitol	61	1.2	0.47
158 (Mannitol "revertant")		82	1.1	0.55
	Mannitol	64	0.7	0.58
	Sorbitol	77	0.8	0.45
159 (Wild type)		2.5	0.1	0.01
	Mannitol	1.3	1.0[e]	0.01
	Sorbitol	78	0.7	0.42
238 (Mannitol constitutive)		1.3	1.2[e]	0.01
	Sorbitol	84	3.3[e]	0.39

[a] Cells and extracts used to determine uptake and enzyme activities with mannitol as substrate were also used to obtain data presented in this table with sorbitol as the substrate.
[b] Markers not specified are wild-type alleles.
[c] 5×10^{-6} M sorbitol.
[d] 5×10^{-5} M sorbitol.
[e] Activity partially attributable to enzyme II complex$_{mtl}$.

clones which were able to grow on mannitol without regaining enzyme II complex$_{mtl}$ (i.e., those that were not true revertants) exhibited constitutivity in the sorbitol pathway.

To test whether constitutivity in the sorbitol system is common among spontaneous "revertants" of mannitol mutants missing enzyme II complex$_{mtl}$, cells of five other independent *mtlA*$^-$ mutants were plated on agar with mannitol as the sole source of carbon and energy. In each case, about 90% of revertant colonies turned out to be constitutive in the sorbitol system.

The product formed from mannitol under the influence of the sorbitol enzyme II complex is evidently mannitol-1-P, since its further metabolism depends on mannitol-1-P dehydrogenase, and a mutant lacking both enzyme II complex$_{mtl}$ and mannitol-1-phosphate dehydrogenase could no longer be suppressed by constitutivity of the sorbitol pathway in a transduction experiment in which strain 158

served as the donor and 10 mannitol-positive clones were analyzed.

Growth on mixed substrates. The mediation of entry and phosphorylation of mannitol by enzyme II complex$_{sbl}$ is supported also by the results of a physiological experiment in which cells of strains 159 (wild type), 157 (mutant), and 158 (suppressed) were compared in their growth response to dual carbon sources of the following: (medium 1) mannitol and glucose, (medium 2) sorbitol and glucose, and (medium 3) mannitol and sorbitol (Fig. 1-3).

Growth of strain 159 was not diauxic in medium 1, but is in media 2 and 3.

Growth of strain 157, as expected, halted in medium 1 after the exhaustion of glucose. Growth in medium 2 was indistinguishable from that observed with the wild-type strain. In medium 3, however, growth continued until both substrates were exhausted. Thus the presence of sorbitol permitted the utilization of mannitol, but in contrast to the diauxic growth observed with the wild-type strain in this medium, growth of the mutant strain was continuous. The failure of the cells to synthesize new enzyme II complex$_{sbl}$ in the absence of sorbitol would explain the gradual slow-down of growth on mannitol.

The growth pattern of strain 158 differed from both strains 159 and 157. Full utilization of both substrates was achieved in all three media, but in contrast to wild-type cells, growth of the suppressor strain was diauxic in medium 1 (strongly), and 2 (weakly), but not in medium 3.

It might also be noted that, after the exhaustion of sorbitol, growth on mannitol continued exponentially instead of linearly, as observed with strain 157.

Substrate uptake. When cells cultured in media containing dual carbon sources were sampled at different periods for measurement of their ability to take up radioactive mannitol or sorbitol individually at a low concentration of 5×10^{-6} M (which is much lower than that which permitted effective utilization for growth), characteristic patterns were also observed for each strain (Fig. 1-3). For example, the mannitol uptake activities of wild-type cells increased rapidly after inoculation into medium 1, indicating that the presence of glucose did not impede the induction of the enzymes of the mannitol pathway. In contrast, glucose, as well as mannitol, prevented the induction of the sorbitol uptake system.

In the case of the mutant strain 157, the ability of the cells to take up labeled mannitol (at the low concentration employed for uptake studies) was insignificant no matter how they were grown. This was not unexpected in view of the genetic defect in enzyme II complex$_{mtl}$. By reason of the same defect, the induction of the sorbitol uptake system was no longer prevented by mannitol.

In the case of the suppressor strain 158, the ability of the cells to scavenge labeled man-

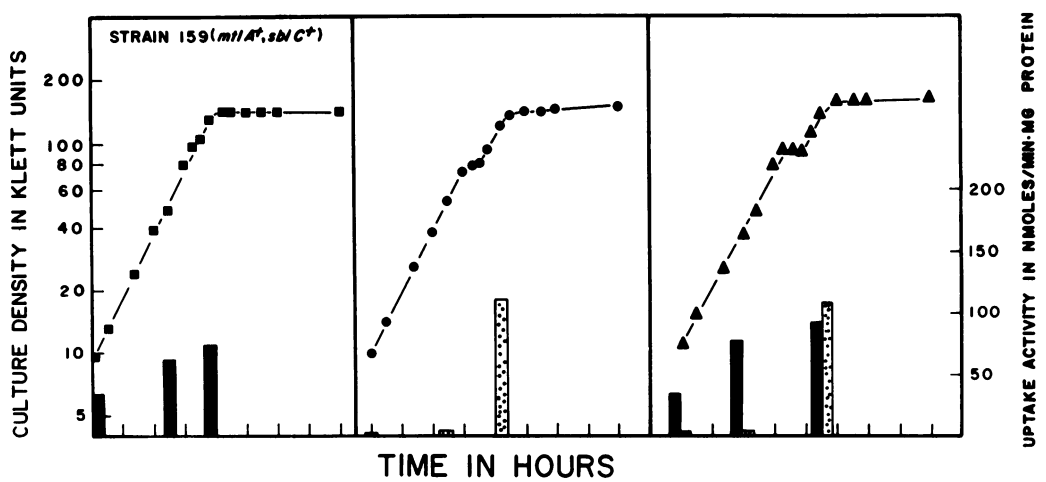

FIG. 1. *Growth responses of strain 159 (wild type) in media containing dual carbon sources. Cells grown overnight on glucose were centrifuged, washed with mineral medium at room temperature, and inoculated in minimal medium containing: mannitol and glucose (■); sorbitol and glucose (●); and mannitol and sorbitol (▲). Solid black bars represent substrate uptake activity by washed cells for ^{14}C-mannitol at 5×10^{-6} M. Stippled bars represent the corresponding uptake activities on ^{14}C-sorbitol.*

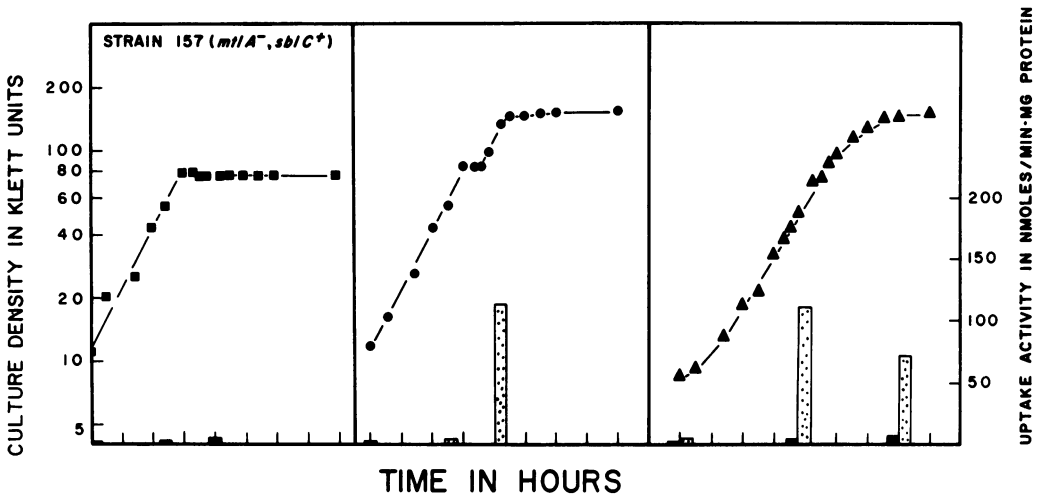

FIG. 2. *Growth responses of strain 157 (mutant) in media containing dual carbon sources. Cells grown overnight on glucose were centrifuged, washed with mineral medium at room temperature, and inoculated in minimal medium containing: mannitol and glucose (■); sorbitol and glucose (●); and mannitol and sorbitol (▲). Solid black bars represent substrate uptake activity by washed cells for ^{14}C-mannitol at 5 × 10^{-6} M. Stippled bars represent the corresponding uptake activities on ^{14}C-sorbitol.*

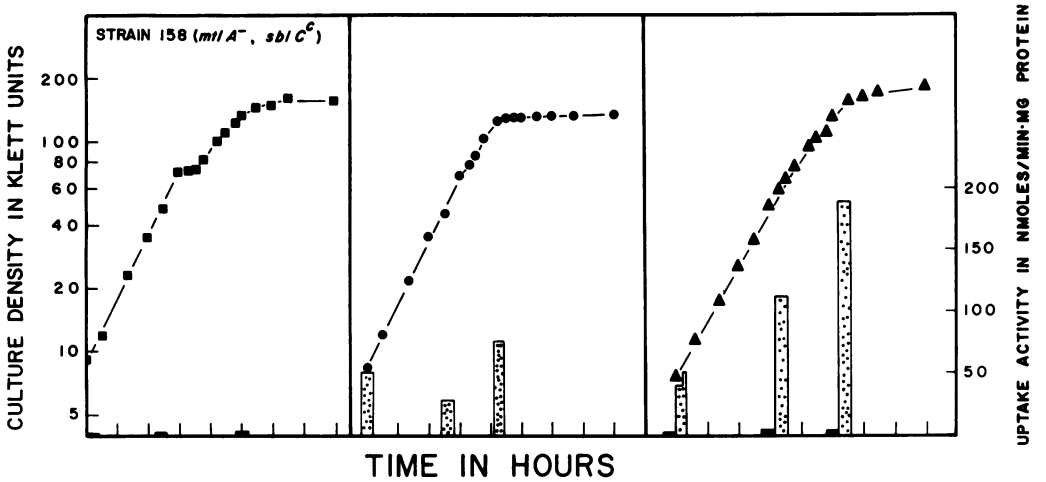

FIG. 3. *Growth responses of strain 158 (suppressed) in media containing dual carbon sources. Cells grown overnight on glucose were centrifuged, washed with mineral medium at room temperature, and inoculated in minimal medium containing: mannitol and glucose (■); sorbitol and glucose (●); and mannitol and sorbitol (▲). Solid black bars represent substrate uptake activity by washed cells for ^{14}C-mannitol at 5 × 10^{-6} M. Stippled bars represent the corresponding uptake activities on ^{14}C-sorbitol.*

nitol remained slight irrespective of the growth condition, like the case of strain 157. The uptake activity for sorbitol of the suppressor strain (*sblC*^{c}), however, was resistant to glucose, in contrast to the two inducible strains.

Mannitol and sorbitol diauxie. The double role of enzyme II complex$_{sbl}$ for the dissimilation of both sorbitol and mannitol in the suppressor strain 158 raises the question of whether the physiological substrate, sorbitol, is preferentially utilized. The order of substrate consumption was therefore compared in strains 159, 157, and 158. From Fig. 4 it may be seen that, whereas wild-type cells utilized mannitol before sorbitol, the reverse was true in strains 157 and 158. This reversal of preference is most probably dictated by the relative affinity of the enzyme II complex$_{sbl}$ for the two substrates.

Confirmation of the MtlA$^-$ SblC^c phenotype. To test whether some of the properties observed of strain 158 might be fortuitous, the

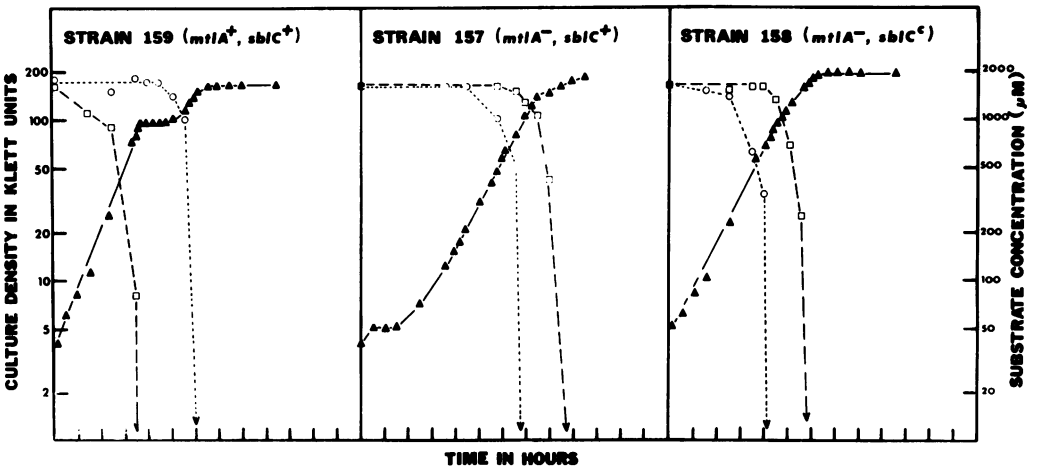

FIG. 4. *Order of mannitol and sorbitol consumption by strains 159, 157, and 158 during growth. Cells grown overnight on glucose were centrifuged, washed, and inoculated in medium containing mannitol and sorbitol. A 1-ml amount of each culture was withdrawn at various intervals. The cells were removed by filtration, and the media were kept frozen until the time of assay of the carbon sources remaining by procedures as described in Material and Methods. Symbols:* ○, *concentration of sorbitol;* □, *concentration of mannitol;* ▲, *cell density. Arrows indicate that the concentration of the carbon source fell below 1 μM.*

sblC^c marker was transduced into another mutant lacking enzyme II complex$_{mtl}$, strain 236 (19), by selection for growth on mannitol. The activities of substrate uptake by intact cells, the enzyme activities in cell-free extracts, and the growth experiments described for strain 158 were completely repeated with the new strain. No significant differences were observed.

DISCUSSION

Several cases are now known in which the loss of a carbohydrate transport system could be at least partially remedied by a suppressor mutation resulting in the constitutive expression of another transport system with overlapping substrate specificity. For example, in *E. coli*, the lost function of a methylgalactoside permease can be replaced by constitutivity of β-galactoside permease (1, 10) or melibiose permease (11; J. Lengeler, *unpublished data*). A strain of *K. aerogenes* which has lost the activity of its enzyme II complex for mannitol can be restored in its ability to grow on this hexitol by constitutivity of the D-arabitol pathway. In this case, probably D-arabitol permease delivers mannitol into the cytoplasm as the free carbohydrate where it is converted to fructose by an NAD-dependent D-arabitol dehydrogenase (21). The intervention of the D-arabitol permease also allows the *K. aerogenes* mutant, selected for growth on xylitol, to scavenge this novel carbon source more effectively (24). A strain of *E. coli* (3, 20) which has lost its enzyme I is enabled to grow on glucose by induction of its methyl-galactoside permease (3, 7). A similar mutant of *Salmonella typhimurium* is enabled to grow by the same means (18). Although glucose is delivered into the cytoplasm by these permeases as a free sugar, subsequent phosphorylation is possible through the intervention of an adenosine triphosphate-dependent kinase (4). In *Bacillus subtilis* the induction of sorbitol dehydrogenase (and presumably also sorbitol permease) endows the cell with the ability to grow on xylitol (6).

In his study of diauxic growth, Monod (15) separated carbon sources into classes A and B according to the preference of their utilization in mixed media. For *E. coli*, both mannitol and glucose belong to class A. No diauxie occurred when cells were presented simultaneously with two class A compounds. On the other hand, when sorbitol, a class B compound, was provided together with mannitol or glucose, diauxie occurred and consumption of sorbitol began only after the class A compound was exhausted. The duration of lag between growth on two carbon sources often could be reduced or eliminated if the cells were either preinduced with the less preferred substrate or presented with it at high concentrations. It was therefore proposed that exclusive utilization of the preferred substrate depended on the prevention of the entry of the inducer of the second catabolic pathway. Subsequent

work in several laboratories (for reviews see 13, 14, 16) revealed that deferment of the utilization of the second carbon source can be imposed by several different mechanisms: (i) interference of transport; (ii) interference with the formation of the inducer; (iii) catabolite and transient repression; (iv) feedback inhibition of a key enzyme.

Results from the present paper show that at least two different mechanisms are involved in the glucose-sorbitol diauxie, both acting at the level of enzyme II complex$_{sbl}$. This is indicated by the fact that although the constitutivity of the sorbitol pathway renders its expression more resistant to glucose, diauxic growth in a medium containing glucose and sorbitol was not entirely abolished but only reduced. The remaining regulatory mechanism can be better seen during growth of strain 158 on a glucose-mannitol mixture. Because the only difference between the suppressed strain and wild-type strain in the mannitol pathway is the substitution of the enzyme II complex, the control must be at this point. In a previous study of diauxic growth of *E. coli* K-12 on glucose and glycerol, it was also found that constitutivity of the glycerol system merely reduced the lag of a diauxic growth. In such cells glucose apparently retains its supremacy by producing fructose-1,6-diphosphate which feedback-inhibits glycerol kinase. Diauxie is abolished only when both the specific repressor system and the feedback mechanism are destroyed (25).

The requisition of a transport system to perform an unusual task illustrates how the order of substrate utilization can be controlled by yet another mechanism. For example, in mutants lacking the enzyme I of the PEP-phosphotransferase system, glucose can share the methylgalactoside permease which normally acts on galactose but has equal affinity for both the hexoses. Under such a condition, glucose and galactose are used simultaneously (7). If, however, in such a cell the β-galactoside transport system is made constitutive, then galactose is used up preferentially (3, 20) because it has two routes of entry, and glucose has only one. The suppression of the mutation in the enzyme II complex$_{mtl}$ by the sorbitol enzyme system provides another illustration of how the order of substrate utilization could be dictated by the affinity of a transport system.

ACKNOWLEDGMENTS

J.L. was supported in part by the Heinrich-Hertz-Stiftung, Düsseldorf. This investigation was supported by grant GB8509 from the National Science Foundation, Public Health Service grant 5 RO1 GM11983 from the National Institute of General Medical Sciences, and grant AHA69-991 from the American Heart Association.

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