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Biochemical and Genetic Study of D-Glucitol Transport and Catabolism in *Bacillus subtilis*

H. CHALUMEAU, A. DELOBBE, AND P. GAY*

Département de Biochimie Cellulaire, Institut de Recherche en Biologie Moléculaire, Centre National de la Recherche Scientifique et Universite Paris VII, 75221 Paris Cedex 05, France

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The catabolic pathway of D-glucitol (sorbitol) in *Bacillus subtilis* Marburg 168M is characterized. It includes (i) a transport step catalyzed by a D-glucitol permease which is affected by the *gutA* mutations, (ii) an oxidation step of the intracellular D-glucitol catalyzed by a D-glucitol dehydrogenase, generating intracellular fructose, affected by *gutB* mutations, and (iii) phosphorylation of the intracellular fructose either at the C1 site or at the C6 site as described previously (A. Delobbe et al., Eur. J. Biochem., **66**:485-491, 1976; A. Delobbe et al., Eur. J. Biochem. **51**:503-510, 1975). Additional data are given concerning the phosphorylation of fructose by a fructokinase (fructose ATP 6-phosphotransferase), which is affected by the *fruC* mutation. The isolation of regulatory mutants affected in *gutR* that synthesize constitutively both the permease and the dehydrogenase indicates the existence of a D-glucitol operon in *B. subtilis*. Unlike the wild-type strain, these mutants are able to utilize D-xylitol as sole carbon source.

A D-glucitol catabolic pathway has been elucidated in *Escherichia coli* by Solomon and Lin (30) and Lengeler and Lin (19). This pathway is initiated by a vectorial phosphorylation, i.e., translocation of the hexitol concomitant with esterification by a phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) as described by Roseman (24). The resulting D-glucitol 6-phosphate is then oxidized by a D-glucitol 6-phosphate dehydrogenase to give fructose 6-phosphate. Mutations affecting each step have been obtained (30, 17), and an extensive study of hexitol transport in *E. coli* by Lengeler (17, 18) provided one of the most precise reports about gene-enzyme relationships in the field of the PTS. Vectorial phosphorylation seems to be the general rule for utilization of D-glucitol by *Enterobacteriaceae*, as indicated by the phenotype of the mutants of *Aerobacter aerogenes* (31) and of *Salmonella typhimurium* (25) lacking the enzyme I of the PEP-dependent PTS (*ptsI*).

We reported previously (6) the existence of another D-glucitol catabolic pathway in *Bacillus subtilis* that involves the production of intracellular fructose (Fig. 1). This pathway was suggested by Horwitz and Kaplan (12) who described the presence of a D-glucitol dehydrogenase activity in this organism (such an activity was described in many other bacteria, including *Pseudomonas sp.* [29] and *Cellvibrio polyoltrophicus* [28]). It was confirmed by the fact that *ptsI* mutants of *B. subtilis* that are unable to transport many carbohydrates (9, 22) can utilize D-glucitol as a sole carbon source. Moreover, we obtained evidence for the presence of a fructokinase (fructose ATP 6-phosphotransferase) which may account for the conversion of intracellular fructose into fructose 6-phosphate (6).

This paper deals with further biochemical and genetic characterization of the D-glucitol catabolic pathway of *B. subtilis*. The first step is catalyzed by a D-glucitol permease which allows the accumulation of intracellular D-glucitol. This transport system is impaired in *gutA* mutants. In both wild-type and *gutA* mutants, D-glucitol can be acted upon by the D-mannitol PEP 1-PTS, but the intracellular D-glucitol 6-phosphate thus formed cannot be catabolized by *B. subtilis*. The second step is catalyzed by the D-glucitol dehydrogenase described previously by Horwitz and Kaplan (12). The third step is a phosphorylation at the C6 site by an ATP-dependent fructokinase. Mutants lacking this enzyme (*fruC*) were utilized previously to characterize the transport role of the fructose PEP 1-PTS (5).

We describe the isolation of mutants that synthesize constitutively both the permease and the dehydrogenase (*gutR*). These mutants are able to utilize xylitol as sole carbon source, whereas the wild-type cannot. They were utilized to characterize the *gutA* and *gutB* mutants, and they allowed the identification of a third class of D-glucitol-negative mutants that lack simultaneously the permease and the dehydro-

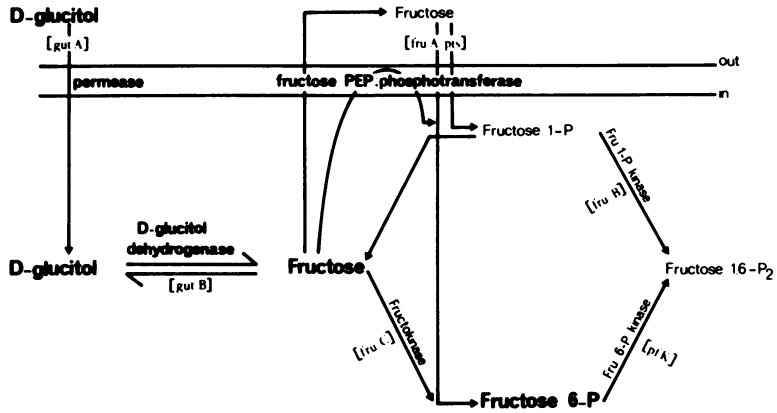

FIG. 1. *Proposed scheme for D-glucitol and fructose catabolism in B. subtilis. The mutations affecting each step are indicated between brackets (10).*

genase. The results suggest strongly that the *gutA* and the *gutB* genes are part of a *gut* operon.

MATERIALS AND METHODS

Chemicals. The following analytical-grade products were used: ATP, oxidized nicotinamide adenine dinucleotide (NAD^+), reduced NAD, and oxidized NAD phosphate were purchased from Boehringer Mannheim Corp. (Paris, France). Glycerol was from Carlo-Erba (Saint-Cloud, France), and D-glucitol, D-mannitol, xylitol, D-fructose, L-arabitol, galactitol, D-gluconate, D-arabinose, and D-glucose were from Merck (Darmstadt, West Germany). D-[^{14}C]glucitol and [^{14}C]mannitol were from New England Nuclear Corp., Boston, Mass. Purified D-glucitol dehydrogenase, yeast hexokinase, 6-phosphoglucose isomerase, and glucose 6-phosphate dehydrogenase were from Boehringer.

Media. The mineral medium C described by Pascal et al. (23) was used for standard cultures and for incorporation tests. The carbon source was usually added at a concentration of 10 g/liter, and nutrients required by auxotrophic strains were supplemented at 50 mg/liter. Sporulation broth (SP medium) was used as a rich medium (27). Solid C medium included purified agar (17 g/liter; Difco Laboratories, Detroit, Mich.), a carbon source (1 g/liter), and auxotrophic requirements (20 mg/liter).

Enzyme assays. The bacteria were suspended in tris(hydroxymethyl)aminomethane (Tris)-chloride buffer (0.1 M, pH 7.5) at 0°C, and sonically disrupted with an ultrasonic MSE disintegrator at 20 KHz. D-Glucitol dehydrogenase was assayed by the procedure of Horwitz and Kaplan (12) in Tris buffer at pH 9.5. The fructokinase assay of Sapico et al. (26) was modified, taking into account some data presented in this paper: 0.1 M Tris buffer (pH 7.7)–0.5 mM fructose–10 mM ATP–10 mM $MgCl_2$. All the assays were carried out with a Gilford recording spectrophotometer. Protein concentrations were measured by the Bücher turbidimetric method (3) with bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) as a standard. Specific activities of permease or enzyme are expressed in nanomoles per minute per milligram of bacterial proteins.

Uptake of ^{14}C-labeled D-glucitol. The kinetics of uptake of radioactive D-glucitol by cell suspensions were followed by the procedure of Delobbe et al. (7). The screening of transport mutants was by the technique of uptake in solid medium (9).

Chromatographic analysis of labeled compounds. The products resulting from the uptake of radioactive D-glucitol were subjected to paper chromatography (paper, Schleicher and Schüll 2316 washed) in solvent 1 (*n*-butanol–acetic acid–water in the ratio 4:1:1 by volume) or in solvent 2 (1 M ammonium acetate [pH 3.8]–95% ethanol [2:5 vol/vol]). The radioactive spots were eluted by distilled water and then identified by enzymatic treatment or by cochromatography with authentic compounds.

Bacterial strains. The strains used are listed in Table 1. They were kept either frozen at −70°C in the culture medium with addition of glycerol (15% final concentration) or as spores on AK (Baltimore Biological Laboratory, Cockeysville, Md.) slants. The nomenclature *gutA*, *gutB*, and *gutR* was chosen to designate the genes involved in the specific steps of the D-glucitol catabolism. According to Lengeler (17), it replaces the nomenclature *sorA*, *sorB*, and *sorR* cited previously (10). Only the relevant genotypes are presented in the text. The symbols Xyl^+/Xyl^- and Gut^+/Gut^- refer to the ability to utilize xylitol or D-glucitol, respectively, as sole carbon source.

Mutagenesis. Mutagenesis experiments were performed by treatment of *B. subtilis* cultures with 0.15 M ethylmethanesulfonate (EMS) (final concentration) for 1 h at 37°C. The washed cells were allowed to grow in SP broth before being plated on C plates supplemented by a mixture of D-glucitol (1 g/liter) and glycerol (0.1 g/liter). The resulting small colonies were patched on glycerol C plates and checked for the D-glucitol phenotype. All the mutants displaying the same phenotype retained in our collection were obtained from mutagenesis of separate clones.

RESULTS

Characterization of a D-glucitol per-

TABLE 1. *List of the bacterial strains mentioned*

Strain no.	Genotype	Origin[a]
168M	*trpC2*	C. Anagnostopoulos
PG500	*trpC2 hisA1*	Multiple-step transformation of 168M
PG515	*trpC2 leu-9 metC3*	Multiple-step transformation of 168M
PG563	*gut-1 hisA1 trpC2*	EMS on PG500
PG593	*gutB1 hisA1 trpC2*	EMS on PG500
PG595	*gut-1 metC3 trpC2*	PG563 $\xrightarrow{tf}$ PG515
PG596	*gutB1 metC3 trpC2*	PG 593 $\xrightarrow{tf}$ PG515
PG602	*fruC1 metC3 trpC2*	Delobbe et al. (6)
PG619	*gutR1 hisA1 trpC2*	EMS on PG500
PG649	*gutR1 gutB1 ura-3*	PG593 $\xrightarrow{tf}$ PG641
PG655	*gutR1 gutA2 hisA1 trpC2*	EMS on PG619
PG656	*gutR1 gutB2 hisA1 trpC2*	EMS on PG619
PG659	*gutR1 gut-7 hisA1 trpC2*	EMS on PG619
PG662	*gutA2 trpC2 leu-8*	PG655 $\xrightarrow{tf}$ PG515
PG668	*gutB2 trpC2 leu-8*	PG656 $\xrightarrow{tf}$ PG515
PG679	*gutR1 gutA9 hisA1 trpC2*	EMS on PG619
PG700	*mtlA1 fruB138 fruC1*	Gay (unpublished data)

[a] X $\xrightarrow{tf}$ Y refers to a transformation cross where X acted as the DNA donor strain.

mease. The occurrence of a specific D-glucitol transport system in the wild-type 168M was suggested by the inducibility of the uptake of the ^{14}C-labeled hexitol (Table 2). This was confirmed by the isolation of mutants lacking the D-glucitol dehydrogenase (*gutB*) which retained this inducible uptake and of a double mutant *gutB gutR* which synthesized constitutively this transport system (Table 3). Figure 2 presents the uptake of D-[^{14}C]glucitol as a function of time in the strain PG596 (*gutB1*). The chase experiment indicated that the plateau observed in the kinetics of the uptake of D-glucitol corresponds to an equilibrium between the efflux and the influx ($V_{in} = V_{ex}$). Furthermore, this plateau showed that the intracellular product of the transport was not metabolized in this mutant. Bacterial extracts obtained from cells of strain PG596 after 10 min of uptake of D-[^{14}C]glucitol were subjected to paper chromatography in solvent 1. Radioautography revealed only one spot which migrated at the same R_f as the D-glucitol added as control. Moreover, the eluted product was converted to fructose by action of commercial D-glucitol dehydrogenase in the presence of NAD^+. These results show that the D-glucitol transport delivers unmodified hexitol inside the cells.

Figure 3 shows the kinetics of D-glucitol uptake in the *gutB* mutant PG596 measured at various concentrations of the substrate. Approximate values of the initial rates of uptake as well as the values of the intracellular concentrations corresponding to each plateau were plotted in a Lineweaver-Burk representation. The same K_m value (0.8 mM) was obtained from the two determinations. The V_{max} was 145 nmol/min per

TABLE 2. *Inducibility of the activities involved in the D-glucitol catabolic pathway*[a]

Inducer	Sp act of:		
	D-Glucitol permease	D-Glucitol dehydrogenase	Fructose-kinase
D-Glucitol	145	180	44
D-Mannitol	20	<1	48
D-Fructose	<1	<1	51
D-Glucose	<1	<1	41
Sucrose	<1	<1	44
D-Gluconate	<1	<1	ND[b]
L-Gluconate	<1	<1	ND
Xylitol	<1	<1	ND
L-Arabitol	<1	<1	ND
Glycerol	<1	<1	55

[a] The strain 168M was grown in C medium supplemented with a mixture of potassium succinate and glutamate and the inducer tested. Specific activities (nanomoles per minute per milligram of protein) were measured as indicated in the text.

[b] ND, Not done.

TABLE 3. *Activities of the D-glucitol pathway in various mutants*

Strain	Carbon sources added in C medium	Sp act of[a]: D-Glucitol permease	D-Glucitol dehydrogenase	Fructose kinase
PG602 (*fruC1*)	SK	<1	<1	<1
	SK + D-glucitol	120	172	<1
PG619 (*gutR1*)	SK	78	184	ND
PG659 (*gut-7 gutR1*)	SK + D-glucitol	<1	<1	48
PG655 (*gutA2 gutR1*)	SK	4	170	ND
PG662 (*gutA2*)	SK	<1	<1	ND
	SK + D-glucitol	3	175	ND
PG656 (*gutR1 gutB2*)	SK	105	<1	ND
PG668 (*gutB2*)	SK	<1	<1	ND
	SK + D-glucitol	89	<1	ND

[a] SK stands for a mixture of potassium succinate and glutamate as carbon source. D-Glucitol was added as inducer. Specific activities (nanomoles per minute per milligram of proteins) were measured as indicated in the text. ND, Not done.

mg of protein. The maximal intracellular concentration based on a bacterial volume of 3.6 ml/g of dry bacteria reached 74 mM. The action of metabolic inhibitors on the uptake was tested; sodium azide (10 mM), 2,4-dinitrophenol (2 mM), and potassium cyanide (10 mM) lower the plateaus of incorporation to 40, 35, and 10% of the control value, respectively.

D-Mannitol-induced D-glucitol uptake. As shown in Table 2, a D-glucitol transport activity was detected in the wild-type strain after growth in mannitol medium. In this case the maximal rate of uptake was less than one fifth of the D-glucitol-induced transport activity. Moreover, a plateau was reached after 15 min of uptake, suggesting that the product accumulated cannot be metabolized. This transport was also detected in a mutant which lacks both D-glucitol permease and D-glucitol dehydrogenase (strain PG595). The labeled intracellular compounds resulting from D-[^{14}C]glucitol uptake by the doubly blocked strain was subjected to paper chromatography in solvents 1 and 2. The radioautography revealed only one spot corresponding very likely to D-glucitol 6-phosphate. Contamination of the commercial D-glucitol by mannitol was excluded because radioactive mannitol should have been metabolized to a nonmigrating radioactive spot. Moreover, low concentrations of D-mannitol drastically inhibit the transport. Finally, no D-glucitol transport occurred in a mutant lacking the enzyme II of the mannitol PTS [strain PG700 (*mtlA1*)] (Gay, unpublished data) grown in C medium containing potassium succinate and glutamate as carbon sources and

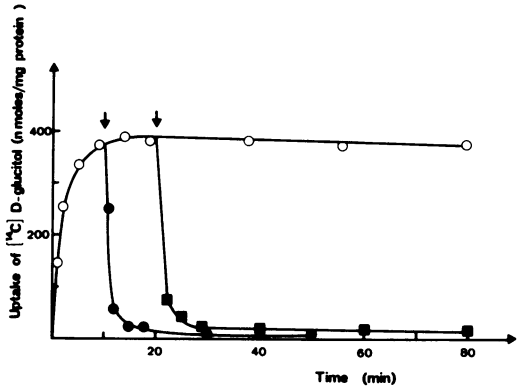

FIG. 2. *D-Glucitol uptake and chase experiment. Cells of strain PG596 (gutB1) were grown in C medium supplemented with a mixture of potassium succinate and glutamate (5 g/liter each) and D-glucitol (5 g/liter). They were allowed to take up D-[^{14}C]glucitol (1 mM, 50 cpm/nmol)* (○). *After 11 min* (●) *and 21 min* (■) *unlabeled D-glucitol (50 mM, final concentration) was added.*

mannitol as inducer. All these data indicated that the enzyme II complex of the PTS for mannitol was responsible for the weak uptake of D-glucitol in cells induced by mannitol.

D-Glucitol oxidation step. Horwitz and Kaplan (12) showed that D-glucitol dehydrogenase catalyzes the conversion of D-fructose to D-glucitol, of xylitol to D-xylulose, and L-sorbose to L-iditol. According to McCorkindale and Edson (21), the enzyme belongs to the class of L-iditol dehydrogenases. We extended this investigation and showed that neither L-arabitol nor

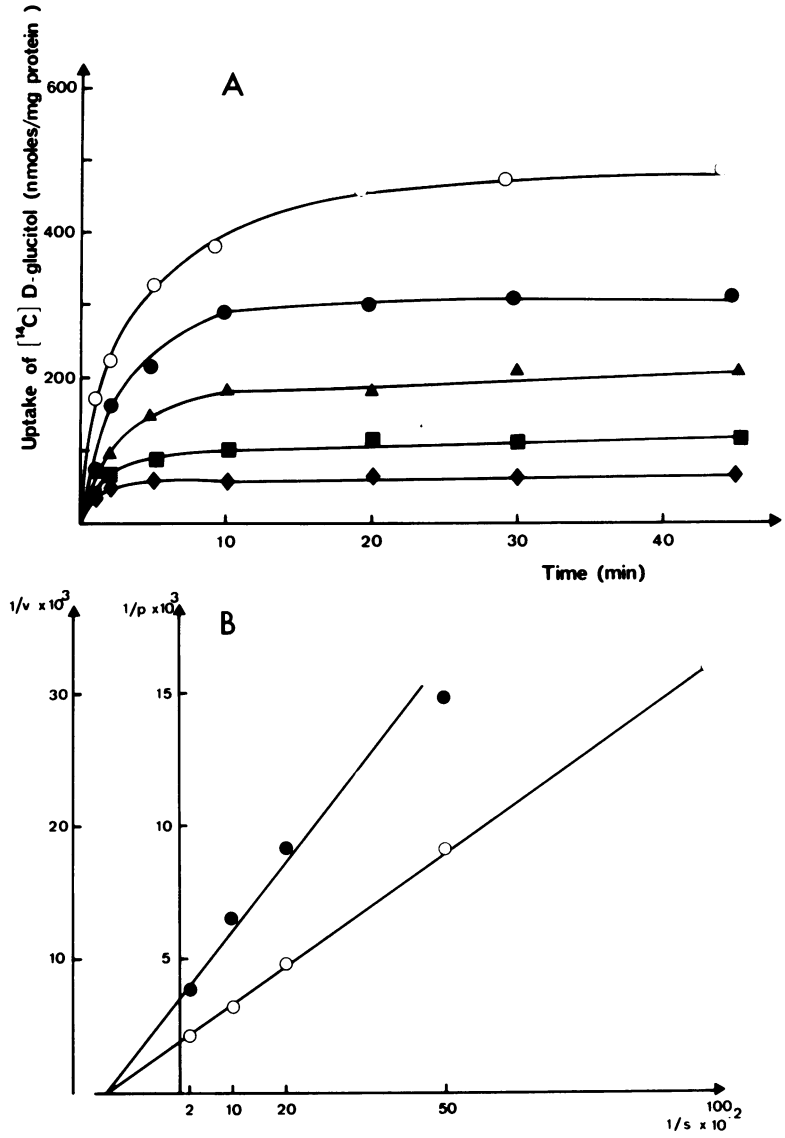

FIG. 3. *D-Glucitol uptake as a function of its concentration in the medium. Cells of strain PG596 were grown in the same conditions as in Fig. 2. The bacteria were allowed to take up radioactive* D-*glucitol at the following concentrations:* ○, *5 mM;* ●, *1 mM;* ▲, *0.5 mM;* □, *0.2 mM;* ◆, *0.1 mM. (A) Kinetics of uptake. (B) Lineweaver-Burk plots of the values of the initial velocities. V (expressed as nanomoles per minute per milligram of proteins) and of the intracellular* D-*glucitol concentration at the plateau, P, are expressed in the same units.*

galactitol was oxidized. D-Gluconate and L-gulonate, the hydroxyl groups of which are in the same steric disposition as iditol, were not substrates of the *B. subtilis* D-glucitol dehydrogenase. Some kinetic parameters of the enzyme are listed in Table 4.

We also extended the study of the inducibility of the enzyme. As shown in Table 2, among the compounds tested only D-glucitol induced the synthesis of the enzyme. D-Mannitol, which induced D-glucitol transport, was ineffective. Xylitol, which is a substrate of the dehydrogenase, cannot induce its synthesis. These regulation properties will be discussed with the help of the genetic data described below.

Phosphorylation of intracellular fructose. As described previously (6) the phosphorylation of intracellular fructose is not a specific

step for D-glucitol catabolism because intracellular fructose may result either from D-glucitol oxidation, from sucrose hydrolysis, or from fructose phosphate hydrolysis. It may follow two alternative pathways. In the wild-type 168M strain fructose is phosphorylated at the C6 site by an ATP-dependent fructokinase. In a mutant lacking the fructokinase (*fruC*), the intracellular sugar is excreted in the medium and is then recaptured by the PEP 1-fructose phosphotransferase. A mutant lacking the phosphofructokinase activity (*pfk*) (10, 8) can utilize fructose as a sole carbon source. It is unable to utilize either D-glucitol (Gut^- phenotype) or a mixture of glycerol and D-glucitol (Gut^s phenotype) because it is inhibited by all of the substrates, the metabolism of which generates fructose 6-phosphate. A double mutant, *pfk fruC*, which does not synthesize fructose 6-phosphate, is not inhibited and is then able to utilize D-glucitol through the fructose 1-phosphate pathway. This property allowed the screening of a large number of *fruC* mutants (10).

As shown in Table 2, the fructokinase activity (fructose ATP 6-phosphotransferase) is synthesized constitutively by *B. subtilis*, i.e., is not subject to the same regulation as the enzymes described above. We found that the activity was optimal at pH 7.7 The K_m for ATP is 0.5 mM. High fructose concentrations inhibit the activity (Fig. 4). A V_{max} of 83 nmol/min per mg of protein and a K_m for fructose of 0.15 mM were obtained with crude extracts.

TABLE 4. *Kinetic parameters of the D-glucitol dehydrogenase*

Determination	D-Glucitol (mM)	Xylitol (mM)
K_m	5	5
V_{max}	170	200
K_m (NAD^+)	0.4	0.66

Mutations affecting the specific steps of the D-glucitol catabolism. (i) Constitutive mutants. The major properties of the mutants described here are listed in Table 3.

Horwitz and Kaplan (12) noted that *B. subtilis* was unable to utilize xylitol when presented as a sole carbon source but was able to use this compound in the presence of D-glucitol. Such a result led us to expect that mutants with constitutive synthesis of the enzymes involved in the D-glucitol catabolism should be able to utilize xylitol as a sole carbon source (phenotype Xyl^+). Spontaneous or EMS-induced mutants that grow on xylitol plates could indeed be obtained. A total of 21 clones, including strain PG619, were checked for xylitol utilization in liquid cultures and for the D-glucitol permease and dehydrogenase activities. The mean generation time in C medium + xylitol was about 140 min. All of the mutants synthesized constitutively both the permease and the dehydrogenase when grown on succinate and glutamate as carbon sources or in SP broth. The mutations designated *gutR* could be transferred to reference strains by DNA-me-

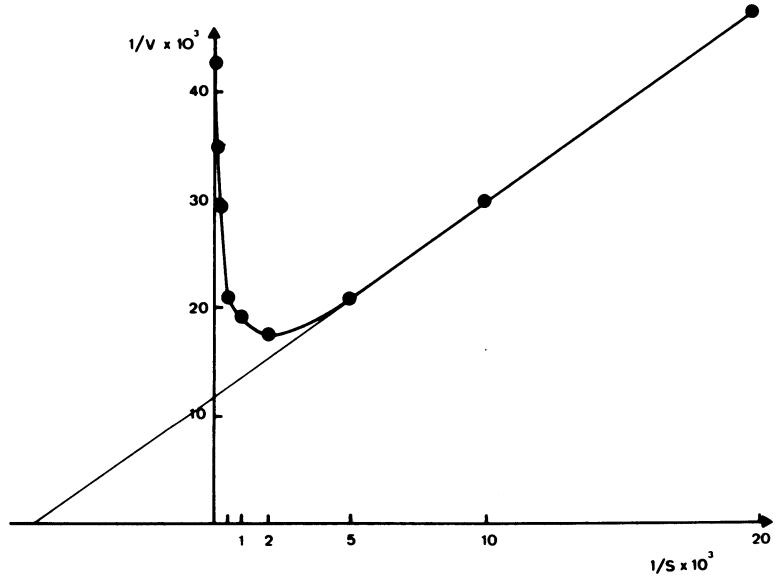

FIG. 4. *Fructokinase activity as a function of fructose concentration. Fructokinase was assayed in a crude extract obtained from the wild-type 168M strain, grown in C medium supplemented with glycerol.*

diated transformation with the same frequencies as known point mutations such as *trpC2* or *hisA1*. The recombinants exhibited the same properties as the original mutants. The existence of the *gutR* mutants provided us with a good tool to analyze the D-glucitol-negative mutants, especially those that lack the permease and which make them behave like regulation mutants.

(ii) D-Glucitol-negative mutants. Among the D-glucitol-negative mutants isolated from the wild-type strain PG500, some lack both the permease and the dehydrogenase activities. It was impossible to tell if this double defect was due to a regulatory or a polar mutation or to a mutation that abolished the permease activity necessary for the entry of the inducer. One of the *gutR* mutants, strain PG619, was therefore used to screen for Gut^- mutants.

The mutations described here are responsible for a Gut^- phenotype. They all revert spontaneously to the parental phenotype. They were transformed to the wild type at the same frequencies as reference markers like *trpC2*, *hisA1*, or *leu-8*, and they were transferred to reference strains at the same frequencies as the latter markers by double transformation. These properties indicated that the Gut^- phenotype studied was very likely due to point mutations.

(iii) D-Glucitol dehydrogenase-negative mutants. Strain PG656 (*gutR1 gutB2*) (as well as strain PG596 [*gutB1*] isolated from the wild-type strain) lacked only the D-glucitol dehydrogenase activity. It was able to take up radioactive D-glucitol because the transport activity was constitutive. However, this double mutant was not able to utilize xylitol as sole carbon source, which indicates that the oxidation of the pentitol is catalyzed by the D-glucitol dehydrogenase.

(iv) Transport mutants. Two classes of mutants unable to carry out the uptake of radioactive D-glucitol were isolated. The first class, including strain PG655 (*gutR1*, *gutA2*), synthesized constitutively the dehydrogenase. These mutations, designated *gutA*, likely affected the structural gene coding for the permease. The other class was devoid of both the permease and the dehydrogenase, like the strain PG659 (*gut7 gutR1*). The presence of the *gutR1* mutation in this stain was tested by transformation with wild-type DNA as donor. Among the Gut^+ transformants, 50% had the Xyl^+ phenotype, indicating the presence of the *gutR1* mutation in the recipient strain.

(v) Genetic linkage of the *gut* mutations. The latter cross provided evidence for a linkage between *gutR1* and *gut-7* markers. Further analogous crosses involving strains PG649 (*gutR1 gutB1*), PG656 (*gutR1 gutB2*), PG655 (*gutR1 gutA2*), and PG679 (*gutR1 gutA9*) as recipient strains, transformed by wild-type DNA (at the concentration of 0.01 μg/ml), indicated that all these markers are linked. The following distances (recombination units) were found: *gutR1-gutB2*, 41; *gutR1-gutB1*, 38; *gutR1-gutA2*, 59; and *gutR1-gutA9*, 71.

DISCUSSION

The two specific steps of the D-glucitol catabolic pathway of *B. subtilis* have been identified with the help of *gutR*, *gutA*, and *gutB* mutants. These steps are catalyzed by a D-glucitol permease and by a D-glucitol dehydrogenase, the synthesis of which is inducible only by D-glucitol in the wild type.

The *gutR* mutants were screened on the basis of their Xyl^+ phenotype. The double mutants *gutR gutA* or *gutR gutB* were able to grow on neither xylitol nor D-glucitol, thus showing that the two substrates share both the permease and the dehydrogenase activities. Because it is very unlikely that the constitutive *gutR* mutants result from two separate events, we assume that the synthesis of the two enzymes is subjected to the same regulation. The existence of a *gut* operon, including two structural genes *gutA* and *gutB*, is supported by preliminary mapping results which indicate that all the *gut* mutations tested are clustered. The simultaneous loss of both the permease and the dehydrogenase activities in a constitutive mutant might thus be the result of a polar mutation.

Isotopic dilution experiments (5) and the behavior of the *pfk* mutants (10) both indicate that the fructokinase mediates the third step of D-glucitol catabolism in the wild-type strains. This activity which is responsible for the intracellular phosphorylation of fructose is also utilized in the catabolism of sucrose (20) and even in the catabolism of extracellular fructose. The broad role is consistent with the fact that the *fruC* gene is not under the same regulation as *gutA* and *gutB* genes. Fructokinase is inhibited by high fructose concentrations, which probably accounts for the low activities reported previously (11) when the assay was performed according to Sapico et al. (26). This kind of inhibition was often reported for kinases which are assumed to combine sequentially with their substrates (1, 2, 32), first with ATP and then with the substrate. In such a case, high fructose concentrations would promote the prior fixation of fructose on the enzyme, thus impeding its combination with ATP (4). The biological significance of such a reaction mechanism, if any, is not clear. All the *fruC* mutants tested possess a normal glucokinase activity, which implies that the *B. subtilis* glucokinase is highly specific. It is likely that bac-

terial kinases devoted to the phosphorylation of hexoses have a higher specificity than eucaryotic hexokinases (13).

A transport system was described. This activity delivers unmodified hexitol within the cells. The kinetic data showed that it obeys the same laws as those described by Képès and Monod (16) and Képès and Cohen (15) for the bacterial permeases. The intracellular concentrations when the plateaus are reached are proportional to the initial rates of entry. Then the actual concentration inside the bacteria is given by $[Gut_{in}] = [Gut_{in}]_{max} [Gut_{ex}]/(K_m + [Gut_{ex}])$, where $[Gut_{in}]_{max}$ and Gut_{ex} are the maximal D-glucitol concentration inside the bacteria and the D-glucitol concentration in the medium, respectively. The theoretical maximal ratio of extracellular to intracellular concentrations of D-glucitol is given by $[Gut_{in}]_{max}/K_m = 92.5$. Such a concentration gradient should require at least a free energy of 2.9 kcal (ca. 12.1 kJ) per mol of accumulated D-glucitol. It is likely that the permease utilizes the energy generated by respiration because the metabolic inhibitors affect its activity. The pattern of inhibition by the poisons used differs somewhat from the pattern described in the case of the citrate permease by *B. subtilis* (33). In the two cases their action is not really understood.

The efflux, the rate of which is equal to the influx rate when the plateau is reached, is indeed proportional to the intracellular D-glucitol concentration. The K value for this first order kinetics is given by $K = V_{max}/[Gut_{in}]_{max} = [145 \times 10^3]/74 = 2$ ml/min × g of protein.

The diffusion process that could account for such an efflux should allow a symmetrical influx, the rate of which should not be consistent with the Gut^- phenotype of the double mutant *gutA gutR* in the standard medium. Consequently, we assume that this efflux is catalyzed by the permease itself, working with a very low affinity, as postulated by Képès (14) in the case of the galactoside permease of *E. coli*.

D-Glucitol transport may also be mediated by the mannitol PEP PTS. Such a low specificity of the mannitol transport system was encountered in *E. coli* (17, 18), which is able to catabolize the D-glucitol 6-phosphate thus synthesized. This is not the case in *B. subtilis*, which lacks a D-glucitol phosphate dehydrogenase (12). In this organism, D-glucitol may be utilized as a non-metabolizable substrate in the study of the D-mannitol PEP PTS.

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